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Resumen por el autor, Albert Kuntz.

Estudios experimentales sobre la histogénesis del sistema nervioso del simpático.

El autor ha extirpado ó destruído total o parcialmente el sistema nervioso cerebro-espinal en una serie de segmentos o en toda la región del tronco de embriones de rana y pollo, antes de que las células de origen cerebro-espinal hayan comenzado a emigrar hacia la periferia. Se dejó continuar el desarrollo del embrión durante tres a cinco días después de la operación. Un estudio de estos embriones ha puesto de manifiesto los siguientes hechos: 1. La relación genética de los sistemas simpático y cerebro-espinal queda demostrada experimentalmente. 2. Los esbozos de los troncos del simpático y los plexos prevertebrales en ausencia de los ganglios espinalis y raíces dorsales se originan exclusivamente de células de origen medular. La mayor parte de las células que normalmente se incorporan a los ganglios de los troncos del simpático y los ganglios prevertebrales se originan en las porciones intermedias de las paredes del tubo neural. 3. Células de origen medular se incorporan al neurilemma de las fibras eferentes de los nervios espinales. 4. Los plexos esofágico, pulmonar, cardíaco y entérico, con la excepción de la porción aboral del tubo digestivo, están genéticamente relacionados con los vagos. Se originan a expensas de células que avanzan desde los ganglios del vago y las paredes del cerebro posterior a lo largo del trayecto del vago. Una contribución más tardía de las células de los esbozos de los troncos del simpático a estos plexos no es imposible.

Translation by José F. Nonidez
Cornell Medical College, New York

EXPERIMENTAL STUDIES ON THE HISTOGENESIS OF THE SYMPATHETIC NERVOUS SYSTEM

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TEN FIGURES

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INTRODUCTION

Studies on the histogenesis of the sympathetic nervous system involve the fundamental principles governing the differentiation of nervous tissue. By reason of this fact, the literature bearing on the development of the sympathetic system in vertebrates has grown very large. In general, the sympathetic has been regarded as genetically and functionally related to the cerebrospinal nervous system. The investigators who have supported this theory agree in deriving the elements which give rise to the sympathetic from the cerebrospinal nervous system. However, their studies reveal no general agreement either regarding the exact sources of the cells which give rise to sympathetic neurons or the pathways along which these elements are displaced into the primordia of the sympathetic nervous system. On the other hand, there has long been a tendency on the part of

certain investigators to regard the sympathetic as the most primitive part of the vertebrate nervous system, having its prototype in the primitive nerve net of lower invertebrate animals. In keeping with this theory, there have been isolated attempts to demonstrate the independent origin of the sympathetic nervous system by a process of local differentiation of mesodermal elements. This lack of agreement regarding the most fundamental aspects of a problem which is essentially analytic emphasizes the desirability of a more exact method of investigation. Therefore, following extensive studies based on normal embryonic material, the writer has employed the experimental method.

Inasmuch as the literature bearing on the development of the sympathetic nervous system was reviewed by the writer in a recent paper ('20), no systematic review will be attempted at this time. The several papers which have appeared more recently, as well as many of the earlier ones, will be referred to in connection with the data presented in this paper.

MATERIAL AND METHODS

The material upon which this paper is based consists of a series of embryos of the chick and the frog which were early subjected to operative procedures and allowed to live for varying periods following operation. The operative technique employed on the embryos of the chick, as indicated in an earlier paper ('20), consisted in the destruction of tissue by means of electrolysis. The electrodes were applied through a circular opening in the egg-shell which was closed after operation by sealing a piece of mica over it. The egg was then returned to the incubator. This operative technique does not differ essentially from that which was described by Clark ('20) and need not be described in greater detail at this time. The operative procedure to which the embryos of the frog were subjected consisted in the extirpation of portions of the cerebrospinal nervous system by means of careful dissection. This technique has been employed so frequently that a detailed description of it would be superfluous.

The embryos of the chick, with few exceptions, were subjected to operation at or before the close of the second day (forty-eight hours) of incubation. At the close of the second day of incubation the spinal ganglia are not yet fully differentiated and in the posterior portion of the trunk the neural tube is not yet closed. In some instances an attempt was made to destroy just enough tissue along the dorsal aspect of the embryo to insure complete elimination of the neural-crest material and leave the ventral half of the neural tube intact. In others, an attempt was made to destroy sufficient tissue to insure complete elimination of the neural crests and the neural tube throughout a series of segments or the entire postcephalic region. In a third group, the electrode was applied asymmetrically in an attempt to destroy the ventral portion of the neural tube unilaterally, leaving the neural crests and the dorsal portion of the neural tube intact. In a fourth group, an attempt was made to destroy the cells which give rise to the sensory ganglia of the vagi and the portions of the hindbrain from which the vagi arise. It is quite impossible at the time of operation to determine just how much tissue is destroyed. In some instances the desired result was obtained; in others, either more or less extensive destruction of tissue occurred than was intended. Consequently, a series of embryos was obtained in which the destruction of nervous tissue varies in extent from the incomplete elimination of the neural-crest material to the complete elimination of the cerebrospinal nervous system throughout a series consisting of few or many segments.

The rate of mortality among the embryos of the chick which were subjected to operation was relatively high and bore a more or less definite relation to the extent of the destruction of tissue. Nevertheless, a sufficient number of embryos survived to afford ample material for study. The majority of those which survived were killed at the close of the fifth day of incubation.

The embryos of the frog were subjected to operation either before or immediately after the closure of the neural tube. In some the neural crests and the dorsal portion of the neural tube, in others, the entire cerebrospinal nervous system was extir-

pated posterior to the cephalic region. In still others, only a portion of the neural tube in the posterior cephalic region was extirpated in an attempt to eliminate the nuclei of origin and the ganglia of the vagi. In a few instances two tadpoles from which a strip of tissue including the neural crests and the dorsal portion of the neural tube was cut were placed in contact with each other in such a manner that the cut surfaces of the neural tubes were approximated. These tadpoles readily healed together, thus preventing any regeneration of the dorsal portions of the neural tubes. In the other tadpoles which were operated in the same manner, the neural tube became closed dorsally, but neither the neural crests nor any considerable portion of the neural tube which was removed was regenerated. Among the tadpoles which survived the operation, those from which a strip of tissue including the neural crests and only the dorsal portion of the neural tube was cut lived a week or longer. Those in which the entire cerebrospinal nervous system was extirpated in the postcephalic region, as well as those in which an attempt was made to eliminate the vagi, failed to live longer than four or five days following operation. These were also retarded somewhat in their development; consequently, they represent a stage of development somewhat less advanced than their age indicates.

THE GENETIC RELATIONSHIP OF THE SYMPATHETIC TO THE CERE BROSPINAL NERVOUS SYSTEM

In his pioneer work on the development of the sympathetic nervous system, Remak ('47) advanced the theory that the cells which give rise to the sympathetic ganglia are differentiated in situ from mesodermal tissue. This theory was accepted by Goette as early as 1875 and still advocated by him at a much later date. Since Balfour ('77) clearly pointed out that the sympathetic is derived from the cerebrospinal nervous system, investigators in this field, with few exceptions, have supported this view. The older theory of the mesodermal origin of the sympathetic nervous system was revived by Paterson ('90) and Fusari ('92). Among the more recent investigators, few

have given the work of the advocates of the theory of the mesodermal origin of the sympathetic nervous system serious consideration because the genetic relationship of the latter to the cerebrospinal nervous system was regarded by them as an established fact. However, as late as 1912, Camus, a student of Goette, once more revived this theory. Furthermore, it has very recently been advanced in a somewhat modified form by Dart and Shellshear ('21) as an integral part of their "new interpretation of the morphology of the nervous system."

The writer is one of those who have regarded the genetic relationship of the sympathetic to the cerebrospinal nervous system as an established fact. However, in view of the recent arguments to the contrary, and inasmuch as the material on which the present study is based affords conclusive evidence regarding the genetic relationship of the sympathetic nervous system, a brief discussion of this problem at the present time may be justified.

Embryos of the chick and the frog in which the neural crests and the neural tube were destroyed throughout a series of segments by operation before cells of nervous origin had advanced peripherally show complete absence of the primordia of the sympathetic trunks and the prevertebral sympathetic plexuses in these segments. The same absence of the sympathetic primordia may be observed also in segments in which a remnant of the ventral portion of the neural tube persists, even though small ventral nerve-roots including no visceral efferent fibers may be present. These facts are illustrated microphotographically in figures 1 and 2, which are taken from sections of an embryo of the chick (14)¹ which was subjected to operation at the close of the second day and killed at the close of the fifth day of incubation. Figure 1 is taken from a section through a lumbar segment in which the cerebrospinal nervous system is entirely absent. The mesenchymal tissue around the aorta presents a relatively homogeneous appearance. There are no aggregates of cells or condensations of tissue along the dorsolateral aspects of

¹ Figures introduced in this manner indicate the number of the embryo in the experimental series.

the aorta which could be interpreted as the primordia of the sympathetic trunks. Neither do aggregates of cells occur along the ventrolateral aspects of the aorta which could be interpreted as the primordia of the prevertebral sympathetic plexuses. Figure 2 is taken from a section through a lumbar segment somewhat farther cephalad. In this segment a remnant of the ventral portion of the neural tube persists from which a slender ventral nerve-root without visceral efferent fibers has grown out unilaterally. This section shows the same absence of the primordia of the sympathetic trunks and the prevertebral plexuses as the one illustrated in the preceding figure. These sympathetic primordia are absent also in all the intervening sections, although sections through the corresponding segments of normal embryos representing the same stage of development show an abundance of cellular elements both in the primordia of the sympathetic trunks and the prevertebral plexuses. In the embryo from which figures 1 and 2 are taken the primordia of the sympathetic trunks are absent in at least eight successive segments. Other embryos of the chick in the present series show the same absence of the primordia of the sympathetic trunks in all segments in which the peripheral migration of cells of nervous origin was prevented by early operation. If the sympathetic were not genetically related to the cerebrospinal nervous system, but differentiated in situ, the primordia of the sympathetic trunks ought to be present in segments in which the cerebrospinal nervous system is absent as well as in segments in which the latter is normally developed.

Embryos of the frog in which the neural crests and the neural tube were extirpated posterior to the cephalic region before any peripheral migration of nervous elements had taken place also show complete absence of the primordia of the sympathetic trunks, although such primordia are present in unoperated embryos representing the same stage of development. This observation is not new. In embryos of the frog from which a strip of tissue including the neural crests and the neural tube was cut early throughout the greater part of the trunk, Harrison ('04) observed that "distal to the point of termination of the intrinsic fibers of the cord there are no nerves of any description in the

organism except sometimes the ramus lateralis vagi." Hooker ('11) also observed the absence of any peripheral nervous structure in embryos of the frog in which the entire cerebrospinal nervous system was extirpated early.

Weber ('51) described a human fetus of about full term in which brain and spinal cord were absent, but the peripheral nerves, including the sympathetic nervous system, were practically normally developed. Dart and Shellshear have interpreted this and other isolated cases in which peripheral nervous elements were observed, in the absence of the cerebrospinal nervous system, as demonstrations of the mesodermal origin of nervous elements and the development of the sympathetic nervous system independently of the neural tube.

In the same paper in which Weber reported the human fetus referred to above, he also reported the case of a calf which was born with the absence of a portion of the spinal cord beginning at the fifth thoracic segment. Spinal nerves and sympathetic trunks were absent throughout the region in which the spinal cord was absent. Weber also cited two cases, one a new-born calf, the other a new-born pig, reported by Alesandrini ('29, '35) which showed conditions analogous to those observed in the calf reported by himself. In the light of these and similar cases and the results of the experimental work cited above, it must be obvious that in the human fetus reported by Weber and in other cases in which peripheral nervous elements were observed in the absence of the central nervous system, the latter must have undergone degeneration after the peripheral nervous system had arisen. Such cases afford no evidence of value regarding the origin of the sympathetic or any other part of the peripheral nervous system.

Primordia of the sympathetic trunks in the absence of the cerebrospinal nervous system may be demonstrated experimentally. Certain embryos of the chick included in the present series were subjected to operation after the initial migration of nervous elements from the cerebrospinal nervous system had taken place. In these embryos, small primordia of the sympathetic trunks are present in segments in which the spinal

nerves and the neural tube are entirely absent. This condition is illustrated microphotographically in figure 7 which is taken from a section through an anterior thoracic segment of an embryo which was subjected to operation about the close of the second day of incubation. Small primordia of the sympathetic trunks (fig. 7, *sy*) are present, in this embryo, in the absence of spinal nerves and complete or almost complete absence of the neural tube in six successive segments. In the posterior thoracic and lumbar regions, the primordia of the sympathetic trunks are absent in at least seven successive segments.

The peripheral migration of nervous elements is initiated somewhat earlier in the anterior thoracic segments than farther posteriorly in embryos of the chick. Obviously, in the embryo cited above, migration was going on in the anterior, but not in the posterior portion of the trunk, at the time of operation. The presence of the small primordia of the sympathetic trunks in the anterior thoracic segments in the absence of the cerebrospinal nervous system does not indicate the local origin of the former. The experimental evidence here presented can only be interpreted as demonstrating the direct genetic relationship of the sympathetic to the cerebrospinal nervous system.

THE HISTOGENESIS OF THE SYMPATHETIC TRUNKS AND THE PREVERTEBRAL PLEXUSES

The sympathetic trunks

According to the teaching of the older investigators who advocated the theory of the ectodermal origin of the sympathetic nervous system, the ganglia of the sympathetic trunks arise exclusively from cells which become displaced peripherally from the spinal ganglia. Cells which arise in the neural tube had previously been traced along the ventral roots of the spinal nerves and into the sympathetic primordia, but the earliest evidence that cells of medullary origin play a major rôle in the development of the sympathetic nervous system was presented by Froriep ('07), who traced such cells along the paths of the ventral roots of the spinal nerves and the visceral rami into the primordia of the sympathetic trunks in embryos of *Torpedo*

and the rabbit. He expressed the opinion that it is essentially these cells which give rise to the neurons in the sympathetic nervous system. Cajal ('08) expressed essentially the same opinion based on observations made on embryos of the chick. The work of Froriep was attacked by later investigators, notably Held ('09) and Marcus ('09), who still adhered to the older teaching. The writer has since 1909 maintained that cells of medullary origin play an important part in the development of the sympathetic nervous system and that the primordia of the sympathetic trunks receive a large contribution of such cells via the paths of the ventral roots of the spinal nerves. This view is also supported by the work of Ganfini ('11-'18), who published extensive and detailed observations on the development of the sympathetic nervous system in embryos of types of all the classes of vertebrates above the Elasmobranchii. Although supported by exhaustive studies, this view was opposed by Neal ('14), who still maintained that a contribution of cells of medullary origin to the primordia of the sympathetic trunks had not been demonstrated. More recently, Müller ('20) once more traced the cells which give rise to the ganglia of the sympathetic trunks exclusively from the spinal ganglia.

In an earlier paper (Kuntz and Batson, '20), experimental evidence was presented which demonstrates a definite developmental relationship of the ganglia of the sympathetic trunks to the ventral roots of the spinal nerves. In embryos of the chick which were subjected to operation before cells of nervous origin had advanced peripherally, the primordia of the sympathetic trunks arose, in the absence of spinal ganglia and dorsal nerve-roots, exclusively from cells of medullary origin which advanced peripherally along the paths of the ventral roots of the spinal nerves. It was pointed out, further, that the facts observed do not exclude the spinal ganglia as a source from which cells may enter the primordia of the sympathetic trunks under normal conditions. It was also pointed out that the primordia of the sympathetic trunks may be of approximately normal size in segments in which the spinal ganglia and dorsal nerve-roots are absent and the remnant of the neural tube is relatively large,

but that "these primordia are small or entirely absent in segments in which the remnant of the neural tube is small and represents only the most ventral portion of the central nervous system, even though ventral nerve-roots are present."

Further experimental evidence indicates that the majority of the cells which enter the primordia of the ganglia of the sympathetic trunks are derived from those portions of the neural tube which give rise to the lateral cell-columns, and that some cells are also contributed by the spinal ganglia. A further contribution of cells from the motor niduli of the ventral nerve-roots is not precluded, but such contribution, if it occur, must be relatively unimportant. Figures 3, 4 and 2 constitute a progressive series and illustrate well the results which follow the early destruction of the neural crests and increasingly larger portions of the neural tube in embryos of the chick. In the embryo (1) from which figure 3 was taken, the neural crest and less than the dorsal half of the neural tube were destroyed unilaterally throughout a series of segments. In these segments, as illustrated in the figure, the dorsal nerve-roots are absent on the side involved, the ventral nerve-roots are relatively large, and the primordia of the ganglia of the sympathetic trunks are of approximately the same size as those on the opposite side. In the embryo (3) from which figure 4 was taken, the neural crests and in excess of the dorsal half of the neural tube were destroyed throughout a series of not less than six successive segments in the lower thoracic and upper lumbar regions. In these segments, as illustrated in the figure, ventral nerve-roots of nearly normal size and small primordia of the ganglia of the sympathetic trunks are present. In the embryo (14) from which figure 2 was taken, the cerebrospinal nervous system was completely or almost completely destroyed throughout the greater part of the trunk region. At the level of the section illustrated in the figure, a remnant of the ventral portion of the neural tube persists from which a slender ventral nerve-root (fig. 2, *vr*) has grown out. This nerve-root contains no visceral efferent fibers. It contains cells of medullary origin, but none deviate toward the aorta and there is complete absence of the primordium of the correspond-

ing ganglion of the sympathetic trunk. In the first of these cases, the intermediate portion of the wall of the neural tube which normally gives rise to the lateral cell-column was left intact. In this case, the primordium of the sympathetic trunk is of approximately normal size. In the second case, a considerable portion of the tissue which normally gives rise to the lateral cell-column was destroyed. In this case the primordium of the sympathetic trunk is correspondingly small. In the third case, all the tissue which normally gives rise to the lateral cell-column was destroyed. Although numerous cells having their origin in the ventral portion of the neural tube advance peripherally along the slender ventral nerve, none of them deviate from the course of these fibers; consequently, the primordium of the sympathetic trunk is absent. These observations strongly suggest that the major portion of the cells which enter the primordia of the sympathetic trunks are derived from the intermediate portion of the wall of the neural tube which gives rise to the preganglionic visceral efferent neurons.

Further evidence regarding the exact sources of the cells which give rise to the ganglia of the sympathetic trunks is afforded by an embryo of the chick (10) in which the ventral portion of the neural tube was more or less completely destroyed unilaterally throughout a series of segments. In some of these segments the neural crest was destroyed, while in others enough of the neural-crest material was left to give rise to a spinal ganglion of nearly normal size. The dorsal portion of the neural tube is somewhat distorted in all of these segments, but relatively little tissue in this portion was destroyed. Figure 5 illustrates the conditions observed in a section through a segment in which the portion of the wall of the neural tube which normally gives rise to the ventral cell-column was almost or quite completely destroyed unilaterally. The spinal ganglion and dorsal nerve-root also are absent. Some fibers may be traced from the intermediate portion of the wall of the neural tube, which is well preserved in this segment, directly toward the dorsolateral aspect of the aorta into the primordium of the sympathetic trunk (fig. 5, *sy*) which comprises an aggregate of cells quite

as large as that which constitutes the primordium of the sympathetic trunk on the opposite side, although on that side both dorsal and ventral nerve-roots show normal development. Unmistakable evidence of active migration of cells directly from the intermediate portion of the wall of the neural tube on the operated side may be observed in several successive sections through this segment. In the absence both of the spinal ganglion and the ventral portion of the wall of the neural tube on this side, practically all the cells which have entered the primordium of the sympathetic trunk must have advanced peripherally from the portion of the wall of the neural tube which normally gives rise to the lateral cell-column.

Figure 6 illustrates the conditions observed in a section a few segments farther caudad in the same embryo. At this level the major portion of the neural-crest material escaped destruction. The spinal ganglion is represented by an aggregate of cells from which fibers of the dorsal nerve-root emerge. The ventral portion of the wall of the neural tube was less completely destroyed in this than in the segment described above, but the intermediate portion suffered more extensive destruction and contains relatively few cells. A very small ventral nerve-root, doubtless, composed both of somatic and visceral efferent fibers accompanied by few cells, joins the dorsal nerve-root as the latter emerges from the spinal ganglion. Small groups of cells, the majority of which apparently have advanced from the spinal ganglion, are present along the course of the spinal nerve. The primordium of the sympathetic trunk in this segment contains relatively few cells (fig. 6, *sy*) and is markedly less conspicuous than its fellow on the opposite side. The phenomena observed in successive sections in this segment justify the conclusion that cells advance from the spinal ganglia into the primordia of the sympathetic trunks. However, the number of cells in the sympathetic primordium on the operated side in this segment is small as compared with the number of cells in the primordium of the sympathetic trunk on the opposite side and on the same side in the segment considered above. In the latter segment practically all the cells in the sympathetic primordium must

have advanced from the intermediate portion of the wall of the neural tube. These observations strongly suggest that the normal contribution of cells from the spinal ganglia to the primordia of the sympathetic trunks is relatively small.

Embryos of the frog from which a strip of tissue including the neural crests and the dorsal portion of the neural tube was removed dorsally before the neural tube was completely closed and which were killed three to five days later show complete absence of spinal ganglia and dorsal nerve-roots throughout the segments involved. Ventral nerve-roots accompanied by cells of medullary origin, as well as small primordia of the sympathetic trunks, are present in all the segments in which a considerable portion of the neural tube was left intact. The number of cells in the primordia of the ganglia of the sympathetic trunks varies with the size of the remnant of the neural tube in the corresponding segment. As far as the study of the development of the sympathetic trunks was pursued in these embryos, the findings corroborate those in the operated embryos of the chick. Embryos of the frog are less favorable for studies of this character than embryos of the chick by reason of the small number of sympathetic elements normally present. Inasmuch as the embryos of the frog included in this experimental series illustrate no phase of the histogenesis of the sympathetic trunks which is not equally well or better illustrated by the embryos of the chick, the conditions which obtain in the former need not be set forth in greater detail at this time.

The prevertebral plexuses

The direct genetic relationship of the prevertebral sympathetic plexuses to the sympathetic trunks is generally conceded, except by those who advocate the theory of the mesodermal origin of the sympathetic nervous system. As pointed out by the writer in earlier studies, the majority of the cells which enter the primordia of the prevertebral plexuses are cells which become displaced ventrally from the primordia of the sympathetic trunks. Other cells which advance toward the latter primordia along the

paths of the visceral rami turn ventrally and enter the primordia of the prevertebral plexuses without having become incorporated in the cell aggregates which give rise to the ganglia of the sympathetic trunks. Consequently, the sources of the cellular elements in the prevertebral plexuses are identical with those of the cellular elements in the sympathetic trunks. These findings are corroborated by the findings in the experimental embryos in the present series. The evidence that cells advance farther peripherally from the primordia of the sympathetic trunks and become incorporated in the primordia of the prevertebral plexuses is conclusive. Furthermore, the number of cells in the latter, as observed in sections of experimental embryos of the chick (figs. 5 and 6, *pp*), bears a definite relationship to the number of cells in the former; i.e., if by reason of the destruction of a relatively large portion of the neural tube the primordia of the sympathetic trunks receive but a small number of cells, the number of cells which become incorporated in the primordia of the prevertebral plexuses is correspondingly small. Yet in no instance was complete absence of the primordia of the prevertebral plexuses observed in the presence of even very small primordia of the sympathetic trunks in the corresponding segments. On the other hand, the primordia of the prevertebral plexuses do not arise in the absence of the primordia of the sympathetic trunks.

THE NEURILEMMA OF THE EFFERENT FIBERS OF THE SPINAL NERVES.

As observed above, cells of medullary origin advance peripherally along the fibers of the ventral roots of the spinal nerves in embryos of the chick in segments in which but a remnant of the neural tube persists and the primordia of the sympathetic trunks fail to arise. Such cells also advance along the somatic efferent fibers of the spinal nerves distal to the point at which the visceral ramus deviates from the ventral ramus in segments in which the primordia of the sympathetic trunks are present, although the spinal ganglia and dorsal nerve-roots are absent. Such cells may be recognized in portions of the spinal nerves dis-

tal to the visceral rami and distinguished from the cells of the surrounding mesenchyme by the greater size and more elongated form of their nuclei. The cells in this portion of the nerve are identical in appearance with the cells which enter the primordia of the sympathetic trunks. They are also intimately associated with the growing nerve-fibers. Obviously, they are cells which become incorporated in the neurilemma.

The origin of the neurilemma of the efferent fibers of the spinal nerves has been studied by not a few investigators. Kupffer ('94, '95), Hoffmann ('97), Harrison ('01), and Neal ('03) presented evidence, based on studies of selachian and fish embryos, which indicates that the elements which give rise to the neurilemma of the fibers of the ventral nerve-roots are cells of medullary origin which advance peripherally along these fibers. In his experimental studies of the development of the peripheral nerves, Harrison ('04) described the spinal nerves, including only ventral root fibers, which arose in embryos of the frog (*Rana esculenta*) in which the neural crests and the dorsal portion of the neural tube had been extirpated early, as composed of naked fibers; i.e., fibers which are unaccompanied by cellular elements. This finding seemed to justify the conclusion that, in the Amphibia, the neurilemma of both the dorsal and the ventral roots of the spinal nerves is derived from cells which advance from the spinal ganglia. This result of Harrison's experimental work has exerted great influence on subsequent investigations and on the more recent literature bearing on the development of the peripheral nervous system. Therefore, it seems worth while to present here certain evidence afforded by embryos of the frog included in the present series. The naked character of the fibers of the ventral roots of the spinal nerves in the absence of spinal ganglia and dorsal roots was confirmed by Harrison ('06) in the American species *R. sylvatica* and *R. palustris*.

Figure 8 illustrates microphotographically the ventral root of a spinal nerve in an embryo of the frog (9) in which the spinal ganglia and dorsal nerve-roots are absent. The neural crests and the dorsal portion of the neural tube were extirpated posterior to the head by cutting a strip of tissue from the dorsal

aspect of the body when the neural tube was closed anteriorly, but still open posteriorly. This embryo was placed in contact with another which had been subjected to the same operative procedure in such a manner that the cut surfaces of the neural tubes were approximated. The embryos healed together in this position, thus preventing any regeneration of the dorsal portions of the neural tubes. They were killed three and one-half days later. This is essentially a repetition of Harrison's experiments, using embryos of another species (probably *Rana pipiens*). As observed in the figure, the fibers of the ventral nerve-root are not naked, but are accompanied by cells obviously of medullary origin (fig. 8, *mc*). A similar result is illustrated in figure 9, which is taken from a section of an embryo of the frog (6) which was subjected to the same operative procedure as the preceding one, but was not grafted to another embryo. It was killed three days after operation. As observed in the figure the remnant of the neural tube is somewhat asymmetrical, but spinal ganglia and dorsal nerve-roots are absent.

If the writer correctly interprets Harrison's present point of view, he does not maintain that no cells of medullary origin advance peripherally along the fibers of the ventral roots of the spinal nerves in embryos of the frog, but that such migration occurs relatively late in embryos of the species which he studied. In presenting the observations recorded above, there is no disposition on the part of the writer to criticise the work of Harrison, but to point out that in embryos of at least one amphibian species, cells of medullary origin migrate into the ventral nerve-roots early. As pointed out above, some of these cells advance into the sympathetic primordia. The evidence presented in figures 8 and 9 does not demonstrate that any of the cells accompanying the fibers of the ventral nerve-roots become incorporated in the neurilemma. However, the availability of these cells is demonstrated. Furthermore, a careful study of a considerable number of embryos of the frog in which the ventral roots of the spinal nerves are present, but the spinal ganglia and dorsal nerve-roots are absent, failed to reveal spinal nerve-fibers unaccompanied by cells of nervous origin. Such cells are pres-

ent also in the ventral rami distal to the level at which the cells which enter the sympathetic primordia deviate from the course of the latter. Obviously, these cells are destined to give rise to neurilemma; consequently, the conclusion that in this species cells of medullary origin become incorporated in the neurilemma is justified.

THE HISTOGENESIS OF THE VAGAL SYMPATHETIC PLEXUSES

A review of the older literature on the development of the sympathetic nervous system reveals no detailed studies on the histogenesis of the peripheral sympathetic plexuses which are functionally related to the vagi, viz., the pulmonary, the cardiac, and the enteric plexuses. The older investigators quite generally supported the theory that the primordia of these plexuses arise from cells which become displaced peripherally from the primordia of the sympathetic trunks.

As early as 1909, the writer presented evidence, based on a study of embryos of the pig, which indicates that the pulmonary, the cardiac, and the enteric plexuses, except in the aboral portion of the digestive tube, arise from cells of cerebrospinal origin which advance peripherally along the paths of the vagi. This evidence was corroborated by the later studies of the writer based on embryos of types of all the classes of vertebrates above the Cyclostomata. It was further corroborated by the work of Abel ('12) on embryos of the chick. More recently, Müller ('20) traced the cells which give rise to the enteric plexuses in selachian embryos both from the vagus ganglia and the sympathetic trunks. Stewart ('20), whose studies are based on embryos of the rat, states "that part if not all of the nerve cells found in the cardiac, gastric, tracheal, oesophageal, pulmonary, and upper intestinal plexuses are of vagus origin."

The problem of the histogenetic relationships of the vagal sympathetic plexuses which are functionally related to the vagi may be attacked experimentally in two ways. If the cerebrospinal nervous system could be destroyed or extirpated throughout the trunk region before cells of nervous origin have advanced peripherally and the embryo should continue to develop without

primordia of the sympathetic trunks, there would remain no source from which cells of nervous origin could advance into the primordia of the sympathetic plexuses associated with and within the walls of the visceral organs except the vagus ganglia and the walls of the hindbrain. If, in such embryos, the primordia of any of the peripheral sympathetic plexuses should arise, either they would arise by a process of local differentiation or from cells of cerebrospinal origin which advance peripherally along the paths of the vagi. On the other hand, if the primordia of the vagus ganglia and the portions of the walls of the hindbrain from which the vagi arise could be destroyed or extirpated early, these nerves would be eliminated. If such embryos should continue to develop with normal primordia of the sympathetic trunks, but the primordia of the sympathetic plexuses associated with and within the walls of the visceral organs should fail to arise, we would be forced to conclude that these plexuses are genetically related to the vagi, and that they normally arise from cells of nervous origin which advance peripherally along the paths of these nerves.

Both methods outlined above were attempted in embryos of the chick and the frog. The complete elimination of the neural crests and the neural tube throughout the trunk region in embryos of the chick which are subjected to operation as early as the close of the second day of incubation involves the destruction of so much tissue that few embryos survive. Fortunately, as pointed out in an earlier section of this paper, the primordia of the sympathetic trunks fail to arise in segments in which a remnant of the ventral portion of the neural tube persists, even though small ventral nerve-roots without visceral efferent fibers may grow out from it (fig. 2). In one embryo of the chick (14) which was subjected to operation at the close of the second day and killed at the close of the fifth day of incubation, the neural crests were completely and the neural tube almost completely destroyed from the lower cervical to the sacral region. In the upper half of the thorax, the neural tube and spinal nerves are entirely absent. In the lower thoracic segments, an asymmetrical remnant of the ventral portion of the neural tube persists

which is large enough to give rise to small ventral nerve-roots unilaterally. In two or three of these segments, small visceral rami and small sympathetic primordia are also present unilaterally. Neither the primordia of the sympathetic trunks nor those of the prevertebral plexuses are present in the lumbar region. The peripheral migration of cells of cerebrospinal origin was effectively prevented throughout the thoracic and lumbar regions in this embryo, following operation, except unilaterally in a few lower thoracic segments. Nevertheless, the primordia of the esophageal, the pulmonary, the cardiac, and the enteric plexuses are present and apparently contain as many cellular elements as in unoperated embryos representing the same stage of development. Figure 10 is a camera-lucida sketch taken from a section of this embryo at the level of the heart. The stippled areas represent branches of the vagi, the solid areas represent aggregates of cells of nervous origin which are incorporated in the primordia of the oesophageal, the pulmonary, and the cardiac plexuses. Unless these cells were differentiated in situ, they must have advanced peripherally along the fibers of the vagi. Branches of the vagi may be traced along the wall of the stomach and the upper portion of the small intestine. Cell aggregates representing the primordia of the enteric plexuses are present in the wall of the stomach and the intestine as far as vagus fibers can be traced. No aggregates of cells representing the primordia of the coeliac plexus are present. The findings in this embryo demonstrate conclusively that the oesophageal, the pulmonary, the cardiac, and the enteric plexuses may arise in the absence of the primordia of the sympathetic trunks or any pathways except those of the vagi along which cells of cerebrospinal origin could migrate peripherally.

The elimination of the vagi by the destruction of the primordia of the vagus ganglia and the portions of the walls of the hind-brain from which these nerves arise is also a drastic operation which few embryos of the chick survive. This operation was not wholly successful in the hands of the writer. However, one embryo survived in which the portions of the hindbrain from which the vagi arise were successfully destroyed, but some of the

cells which give rise to the sensory ganglia of the vagi escaped destruction. Consequently, small ganglia from which afferent vagus fibers may be traced along the oesophagus are present. This embryo died just before the close of the fifth day of incubation, but was fixed before extensive postmortem changes had taken place. The primordia of the sympathetic trunks and the prevertebral plexuses are present throughout their entire extent. A few cells may be traced from the small vagus ganglia along the fibers which emerge from the distal aspects of the latter, but none have reached the level of the heart or the roots of the lungs. The primordia of the pulmonary, the cardiac, and the enteric plexuses are absent. In unoperated embryos representing the same stage of development, the primordia of the cardiac and the pulmonary plexuses are well established. Cell aggregates representing the primordia of the enteric plexuses are also present in the walls of the stomach and the upper portion of the small intestine. The absence of the primordia of the vagal sympathetic plexuses in this operated embryo disproves conclusively both that the cells which give rise to them are differentiated *in situ* and that cells are early displaced from the primordia of the sympathetic trunks into the primordia of these plexuses. These findings, in conjunction with the fact recorded above that the primordia of the oesophageal, the pulmonary, the cardiac, and the enteric plexuses arise in the absence of the primordia of the sympathetic trunks or any pathways except those of the vagi along which cells of cerebrospinal origin could advance peripherally, demonstrates the genetic relationship of these plexuses to the hindbrain and the vagus nerves. However, the embryo which showed absence of the primordia of the vagal sympathetic plexuses in the presence of the primordia of the sympathetic trunks had not reached the stage of development at which the migration of cells into the sympathetic primordia normally ceases. Therefore, a late contribution of cells from the primordia of the sympathetic trunks to the vagal sympathetic plexuses is not precluded.

As observed above, a portion of the cells which give rise to the sensory ganglia of the vagi escaped destruction in the em-

bryo in which the portion of the hindbrain giving rise to the efferent components of the vagi was destroyed; consequently, small vagus ganglia and some afferent fibers are present. However, but few cells advanced from these ganglia along the growing afferent fibers. This observation is of interest in view of the experimental evidence presented above which indicates that the majority of the cells which become incorporated in the primordia of the sympathetic trunks have their origin in the neural tube and advance peripherally along the efferent fibers of the spinal nerves. It strongly suggests that, likewise, the majority of the cells which become incorporated in the primordia of the vagal sympathetic plexuses arise in the hindbrain and advance peripherally along the efferent fibers of the vagi.

The operative procedure involved in the extirpation of the neural crests and the neural tube in the trunk region or the portions of the hindbrain which give rise to the vagi in embryos of the frog is relatively simple. A goodly number survived the operation and continued to develop at a somewhat retarded rate for several days. None survived longer than five days following operation. If development had not been retarded in these embryos, they would have been sufficiently advanced three or four days following operation to have afforded material from which conclusive evidence regarding the histogenetic relationships of the vagal sympathetic plexuses could have been obtained. Some of these embryos which were killed three to four days following operation afford some data which are worthy of consideration. Those in which the cerebrospinal nervous system was extirpated posterior to the head show complete absence of the primordia of the sympathetic trunks, but cells of nervous origin may be traced along the fibers of the vagi into proximity with the heart and along the walls of the oesophagus as far as the growing fibers have advanced. Those in which the vagi were eliminated by extirpation of the corresponding part of the hindbrain show complete absence of nervous elements associated with the heart and the walls of the digestive tube as far as the latter have become differentiated, although the pri-

mordia of the sympathetic trunks are present. These findings, though not conclusive by reason of the early stage of development represented by the embryos, are in full accord with the findings in embryos of the chick set forth above.

DISCUSSION

The experimental data set forth in the preceding pages prove conclusively that the sympathetic primordia are made up both of cells which arise in the neural tube and the cerebrospinal ganglia. Furthermore, they prove conclusively that the contribution of cells from the neural tube to the primordia of the sympathetic trunks and the prevertebral plexuses is much greater than that from the spinal ganglia. They strongly suggest also that the major portion of the cells coming from the neural tube arises in the intermediate portions of its walls, i.e., the portions which give rise to the lateral cell-columns. Likewise, they demonstrate the genetic relationship to the vagi of the oesophageal, the pulmonary, the cardiac, and the enteric plexuses, except in the aboral portions of the digestive tube, and indicate that the major portion of the cells which enter the primordia of these plexuses arises in the portions of the walls of the hindbrain which give rise to the visceral efferent components of the vagi.

An experimental determination of the exact sources of the cells which give rise to the cranial sympathetic ganglia was not attempted. The writer ('20) has previously presented evidence which indicates that these ganglia arise both from cells which advance from the medullary tube and certain of the sensory cerebral ganglia. Doubtless, the differentiation of nervous tissue is governed by the same fundamental principles throughout the entire body. Therefore, the experimental data regarding the histogenesis of the other portions of the sympathetic nervous system presented in this paper lend support to the view that the cranial sympathetic ganglia also comprise both cells of medullary and ganglionic origin.

The sympathetic system is essentially an efferent system. Doubtless, all the neurons in the ganglia of the sympathetic trunks and the prevertebral plexuses are efferent in character. The

majority of the cells which enter the primordia of these ganglia, as indicated above, advance from the neural tube via the paths of the ventral roots of the spinal nerves. These cells arise in portions of the neural tube which give rise to efferent elements. Doubtless, many of them give rise to sympathetic neurons. On the other hand, some cells become displaced from the spinal ganglia, which are essentially sources of afferent neurons, into the sympathetic primordia. Do these cells also give rise to sympathetic neurons? The present data do not warrant a conclusive answer to this question. Doubtless, many of the cells which enter the sympathetic primordia from the spinal ganglia became incorporated in the neurilemma of the visceral afferent fibers of the spinal nerves. Possibly others give rise to supporting elements in the sympathetic ganglia. The experimental data here presented are not incompatible with the theory that only cells of medullary origin give rise to neurons in the ganglia of the sympathetic trunks and the prevertebral plexuses. However, a definite conclusion on this point awaits further experimentation.

The cardiac and the pulmonary plexuses, doubtless bear the same genetic relationship to the hindbrain as do the sympathetic trunks and the prevertebral plexuses to the spinal medulla.

According to one school of physiologists, the sympathetic nervous system contains no afferent neurons. Nevertheless, local reflexes are known to occur in the walls of the digestive tube in the absence of extrinsic nerves. This fact suggests the presence of afferent neurons in the enteric plexuses. Furthermore, the morphological relationships of the neurons in these plexuses, as described by the present writer ('13) and by Müller ('20), suggest that the enteric plexuses constitute a local reflex mechanism involving both afferent and efferent neurons. If this interpretation be correct, it might logically be assumed that the afferent neurons in these plexuses arise from cells which migrate from the vagus ganglia, while the efferent neurons arise from cells which advance from the walls of the hindbrain.

The homology of the cells of nervous origin which advance into the primordia of the sympathetic nervous system with the

cells which give rise to neurons and supporting elements in the central nervous system was pointed out by the writer in earlier papers. Furthermore, the conclusion was drawn that the sympathetic system is homologous with the other functional divisions of the nervous system. The experimental data presented in this paper also justify this conclusion.

SUMMARY

The genetic relationship of the sympathetic to the cerebro-spinal nervous system is experimentally demonstrated.

The primordia of the sympathetic trunks and the prevertebral sympathetic plexuses arise, in the absence of spinal ganglia and dorsal nerve-roots, exclusively from cells of medullary origin which advance peripherally along the paths of the ventral nerve-roots. The majority of the cells which normally enter the primordia of the sympathetic trunks and the prevertebral plexuses arise in the intermediate portions of the walls of the neural tube. Experimental evidence also indicates that a small quota of cells normally advances from the spinal ganglia into the sympathetic primordia.

In the absence of spinal ganglia and dorsal nerve-roots, the efferent fibers of the spinal nerves, both in embryos of the chick and the frog, are accompanied by cells of medullary origin. Obviously, cells of medullary origin become incorporated in the neurilemma of these fibers.

The primordia of the vagal sympathetic plexuses arise in embryos in which the peripheral migration of cells of cerebro-spinal origin is prevented throughout the trunk region by early operation. They do not arise in embryos in which the vagi were eliminated by early operation, although the primordia of the sympathetic trunks and the prevertebral plexuses are present. These findings warrant the conclusion that the vagal sympathetic plexuses arise from cells which advance from the vagus ganglia and the walls of the hindbrain along the paths of the vagi. However, a late contribution of cells from the primordia of the sympathetic trunks to the primordia of these plexuses is not precluded.

The experimental data presented are not incompatible with the theory that all efferent sympathetic neurons arise from cells which have their origin in the medullary tube. A definite conclusion on this point awaits further experimental work.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Five-day chick embryo, no. 14. Section through lumbar segment ($\times 100$), showing absence both of cerebrospinal nervous system and primordia of sympathetic trunks and prevertebral plexuses.

2 Five-day chick embryo, no. 14. Section through anterior lumbar segment ($\times 120$), showing remnant of neural tube with small ventral nerve-root (*vr*), but absence of primordia of sympathetic trunks and prevertebral plexuses.



PLATE 2

EXPLANATION OF FIGURES

3 Five-day chick embryo, no. 1. Section through thoracic segment ($\times 165$), showing ventral nerve-root (*vr*) and primordium of sympathetic trunk (*sy*) in absence of spinal ganglion and dorsal nerve-root.

4 Five-day chick embryo, no. 3. Section through thoracic segment ($\times 165$), showing remnant of neural tube with ventral nerve-root (*vr*) and small primordium of sympathetic trunk (*sy*).

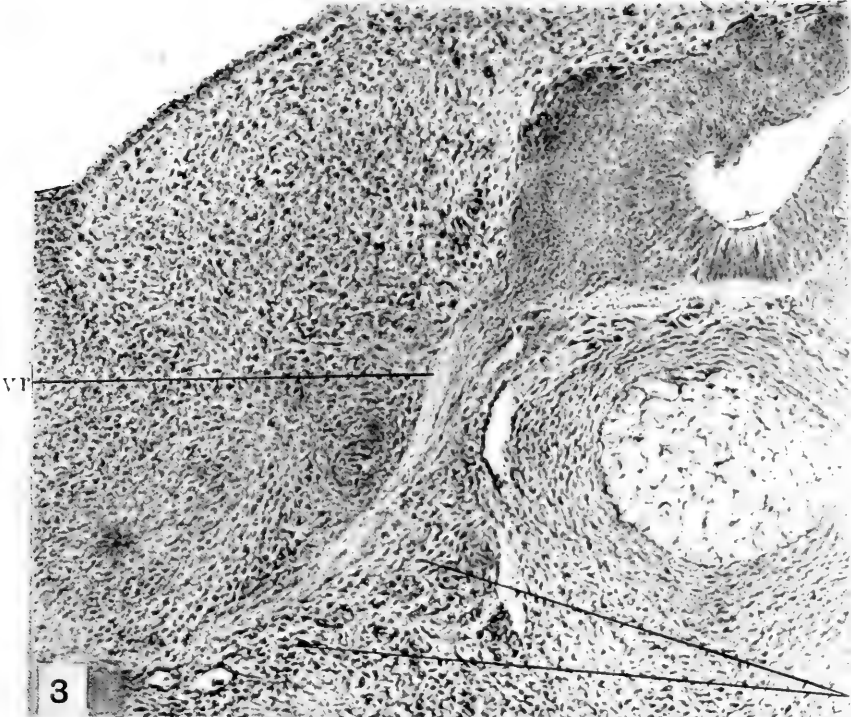


PLATE 3

EXPLANATION OF FIGURES

- 5 Five-day chick embryo, no. 10. Section through lumbar segment ($\times 125$), showing unilateral destruction of ventral part of neural tube, absence of spinal ganglion and dorsal nerve-root, presence of few ventral root fibers and primordium of sympathetic trunks (*sy*) and prevertebral plexuses (*pp*); *mc*, cells migrating from intermediate portion of wall of neural tube.
- 6 Five-day chick embryo, no. 10. Section through lumbar segment ($\times 60$), showing unilateral destruction of ventral part of neural tube, presence of spinal ganglion (*spg*) and dorsal nerve-root (*spn*) and primordia of sympathetic trunks (*sy*) and prevertebral plexuses (*pp*).

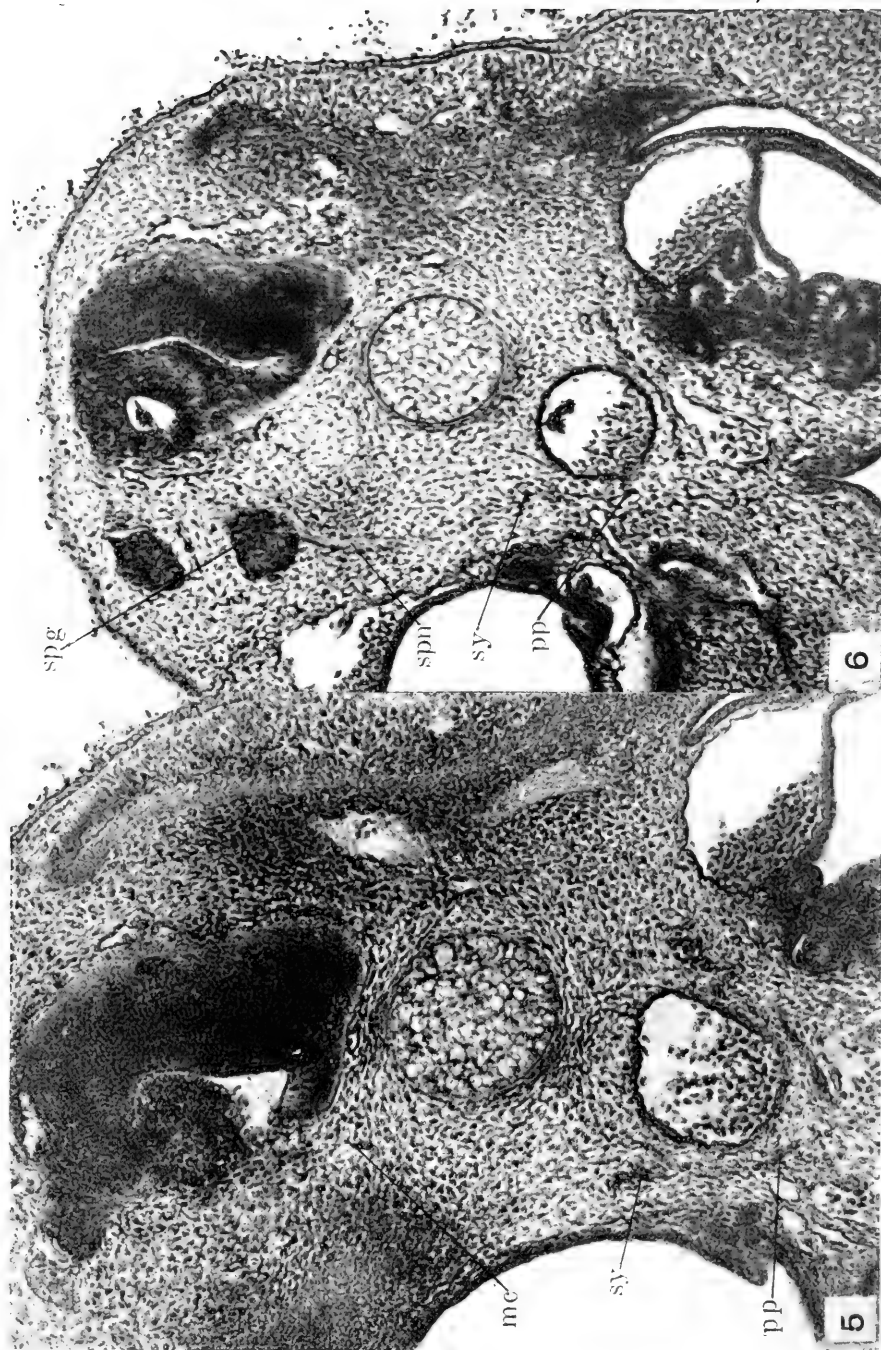


PLATE 4

EXPLANATION OF FIGURES

7 Five-day chick embryo. Section through anterior thoracic segment ($\times 60$), showing small primordia of sympathetic trunks (*sy*) in the absence of spinal nerves and neural tube.

8 Embryo of frog, no. 9, three and one-half days after operation. Section ($\times 275$) showing ventral nerve-root (*vr*) accompanied by cells of medullary origin (*mc*) in absence of spinal ganglion and dorsal nerve-root; *l*, line along which cut surfaces of neural tubes of two embryos healed together.

9 Embryo of frog, no. 6, three days after operation. Section ($\times 365$) showing ventral nerve-root (*vr*) accompanied by cells of medullary origin (*mc*) in absence of spinal ganglion and dorsal nerve-root.



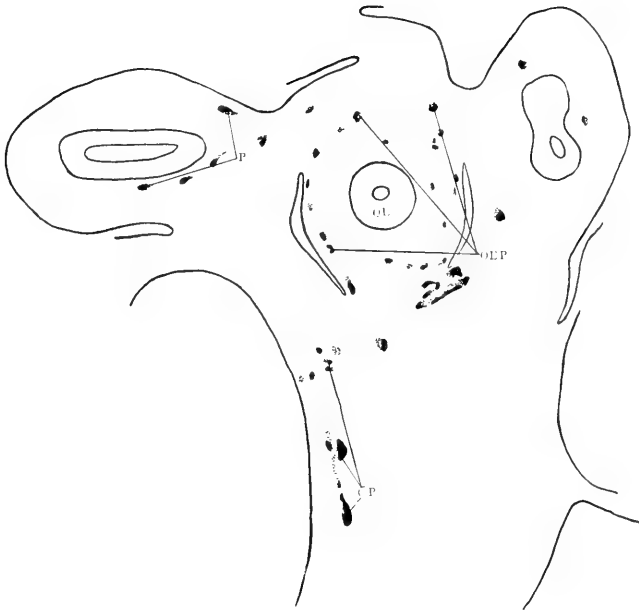


Fig. 10 Five-day chick embryo, no. 14. Camera-lucida sketch from a section at the level of the heart and the roots of the lungs, showing primordia of oesophageal (*OEP*), pulmonary (*P*), and cardiac (*CP*) plexuses; *OE*, oesophagus.

Resumen por el autor, L. S. Ross.

Citología de las grandes células nerviosas del cangrejo de río
(*Cambarus*).

1. El origen intracelular del axon de las grandes células nerviosas del cangrejo consta de un gran número de neurofibeillas ampliamente distribuídas en el citoplasma y casi rodeando al nucleí. Las fibrillas convergen para formar un axon intracelular situado profundamente dentro del cuerpo celular, mientras que en algunos invertebrados y en la mayor parte de los vertebrados las fibrillas convergen en una colina axónica situada cerca de la periferia de la célula. 2. El autor ha obtenido algunas pruebas de la probable existencia de neurofibrillas en el citoplasma vivo, en células teñidas vitalmente. 3. El aparato reticular interno de Golgi no ha sido demostrado. 4. Los granos de Nissl no pueden percibirse en las células vivas, pero aparecen en las fijadas y teñidas. Los granos mencionados son probablemente artefactos, si bien no existe razón alguna para dudar la entidad química de la substancia (cromidial) de dichos granos. 5. Las mitocondrias pueden demostrarse fácilmente en las células y a lo largo del trayecto de las fibras. 6. El trofospongio presenta conezió con las células de la vaina, y puede seguirse como filamentos muy delicados, en sección, los cuales penetran incluso hasta el centro de algunas de las células.

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CYTOLOGY OF THE LARGE NERVE CELLS OF THE CRAYFISH (CAMBARUS)

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FIVE PLATES (TWENTY-ONE FIGURES)

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INTRODUCTION

Since the discovery of mitotic cell division histologists have given much more attention to the nucleus of the cell than to the cytoplasm. This was due in part, and perhaps principally, to the search after the bearer of heredity resulting in the belief that the chromatin is such a bearer. For some time the cytoplasm was more or less neglected, but of recent years it has received more attention, some cytologists insisting that in some degree, at least, it shares with the nuclear material in functioning as a transmitter of heritable characters. Even though this function be denied it, yet its importance is realized because of its relationship to the nuclear material as its immediate environment, and of the interactions between them. The complexity of the structure of the cytoplasm is manifest as a result of studies

NOTE.—I gratefully acknowledge indebtedness to Prof. R. R. Bensley, Prof. C. Judson Herrick, and Prof. G. W. Bartelmez for kindly criticism and valuable suggestion.

of recent years, but problems of its morphology yet remain unsolved.

The cytologist is confronted in his work by some obstacles that at present seem almost insurmountable. It is difficult for him to determine in all cases whether he is observing structures that are present as such in the living cell or whether he is observing artefacts. Do Nissl bodies, neurofibrillae, Golgi internal reticular apparatus, etc., have existence in living undisturbed cytoplasm, or are they the results of violent disturbances caused by reagents? Important as the answers to these questions may be as concerns the ultimate structure of protoplasm, yet is it really of vital importance to the cytologist in comparative work, provided he realizes and recognizes limitations? Does this lack of knowledge of necessity vitiate all his conclusions? That artefacts are produced by reagents is not doubted, but the difficult question is, what appearances are those of artefacts and what ones are not. Certainly, some conjectures based upon various experiments in producing artefacts are not warranted. Unquestionably, reagents produce artefacts in albumen solutions, as reticulations, etc., but comparisons between the effects produced by reagents upon the cell and upon colloidal solutions in a test tube are not wholly justifiable. At best the results from such experiments can give rise to inferences of probability only. As a generalization, however, similarity of results indicates similarity of materials upon which the technic was used. Likewise, dissimilarity of results indicates dissimilarity of materials. The importance of the study of living and postvital, fresh, unfixed cells cannot be overestimated, as apochromatic objectives and the true vital dyes have enabled us to distinguish various elements of the cytoplasm under these conditions.

If the cytologist finds that a particular appearance is produced by the same technic upon cells from the same source, and also by a variation of technic, and if he finds further that cells from different sources respond similarly to the same technic and to variations of technic within limits, then he has a basis for cytological work upon cells of a specific kind and also for comparative cytology—a basis, it is true, that is not so satisfactory

as that of recognized structural elements in the living cytoplasm, but one, nevertheless, upon which a stable scientific superstructure may be erected. If Nissl bodies, neurofibrillae, etc., are made evident in various cells by the same technic, whether these be artefacts or not, the observer is justified in the opinion that there is probably also a similarity between the cells in the living condition.

MATERIAL AND METHODS

The nerve cell of the crayfish serves well as a subject for cytological study. Material is readily obtained and but little work has been done upon it. The ventral ganglia with their large cells are so easy of access that no postmortem changes need occur. Only two or three minutes are needed for the killing of the animal and the removal and transfer of the cord to the fixing solution. The large cells lend themselves especially well to the study of the trophospongium and of the origin of the axis cylinder.

Most of the material used was from young specimens and adult specimens of *Cambarus* soon after they were collected in the field. Practically all of the current neurocytological methods were tried, but only the following yielded results at all satisfactory.

1. Intravital staining

After intravital staining with thionin or methylene blue the neurofibrillae could be clearly seen in the fresh teased material. A .01 per cent aqueous solution of thionin or methylene blue was injected into the pericardial sinus. Upon the death of the animal, twenty to sixty minutes later, the abdominal nerve cord was removed into a thionin solution of the same or double strength and teased. For thirty minutes after removal no postvital changes were usually noted so that there was opportunity to study the cells with some care.

2. Silver methods

All of the silver methods proved to be exceedingly fickle. There were striking differences between the reactions obtained in young animals 4 to 5½ cm. long, and those obtained in adults.

For neurofibrillae the most satisfactory results were obtained by the Cajal procedure used by Boulé ('07) for *Lumbricus* which I have modified only by using formol neutralized with magnesium carbonate instead of adding ammonia to the commercial solution. The method employed by Cowdry ('12), which involves fixation in the 6:3:1 mixture of Carnoy followed by silver impregnation and reduction with pyrogallol, and the Ranson pyridine-silver method were used also.

For the internal reticular apparatus of Golgi the original Golgi technic and the Kopsch and Cajal methods were tried, but nothing was obtained in the large ganglion cells which could be interpreted positively as this apparatus.

3. General histological methods

In some respects the most striking results were obtained by the use of Bensley's ('11) 'A. O. B.' method. The mitochondria are preserved and stained, the Nissl substance can be distinguished, and the intracellular axone is sharply differentiated with the individual fibrillae distinct. The procedure is as follows:

1. Fix 2 to 4 hours in:
 - Potassium bichromate, 2.5 per cent, 16 cc.
 - Osmic acid, 2 per cent, 4 cc.
 - Acetic acid, two small drops.
2. Distilled water, few minutes.
3. Grades of alcohol to absolute, 2 to 4 hours each.
4. Alcohol 100 per cent and bergamot oil, half and half, 1 hour.
5. Bergamot oil, 1 to 2 hours.
6. Bergamot oil and paraffin, half and half, 1 hour.
7. Paraffin, 60°C., 2 hours. Section 4 μ ; fix to slides.
8. Remove paraffin with toluol or xylol.
9. Alcohol 100 per cent down through grades to distilled water.
10. Potassium permanganate 1 per cent, 15 to 30 seconds.
11. Oxalic acid 5 per cent, 15 to 30 seconds.
12. Stain 4 to 5 minutes at 60°C. in Altmann's anilin fuchsin:
 - Anilin water, 100 cc.
 - Acid fuchsin, 20 grams.
13. Rinse in distilled water.
14. Methyl green or toluidin blue 1 per cent solution or Wright's blood stain, a few seconds to be determined by trial.
15. Drain, dehydrate in alcohol 100 per cent, usual procedure for mounting.

For tissue masses larger than crayfish ganglia it may be necessary to extend the time in the process through no. 7.

For neurofibrillae a modification of the method of Donaggio ('05) proved very serviceable. It is as follows:

1. Fixation, 24 hours in Heidenhain's corrosive sublimate.
Remove excess of sublimate with iodine solution.
2. 2 to 3 hours in distilled water.
3. 48 hours in pyridine, changing pyridine after 24 hours.
4. 24 hours in distilled water, changed frequently.
5. 24 hours in aqueous solution ammonium molybdate, plus 1 minim hydrochloric acid to 1 gram of molybdate. Solution should be fresh. (I used ammonium pierate in place of molybdate.)
6. 24 hours in distilled water, changed a few times.
7. Dehydrate and imbed. Usual procedure.
8. Stain thin sections, 4 to 5 μ , in 0.01 per cent thionin.
(I stained overnight. Stain is removed rather rapidly in dehydration.)

For the study of the Nissl substance various additional fixatives were used. Heidenhain's corrosive sublimate solution, formol bichromate (Regaud-Cowdry), acetic-osmic-bichromate (Bensley) and 2 per cent aqueous osmic acid show distinct Nissl bodies with irregular clear spaces between them. Formol neutralized with magnesium carbonate or the 6:3:1 mixture of Carnoy show bodies, but they are not so clearly defined. Whether this is due to a different behavior of the stain or to the effect of the fixatives was not determined.

All material was imbedded in paraffin and cut 3 to 7 μ in thickness.

LITERATURE

Relatively little work has been done on the cytology of the crayfish nerve cell. Holmgren's theory of the 'trophospongium' was based primarily on his observations of *Astacus* as well as *Lophius* cells. His interpretation of the former I have discussed in a paper (Ross, '15) in which I suggested confining the term 'trophospongium' to the capsular septa which extend into the cytoplasm. In the comprehensive paper of 1900 Holmgren figured a large nerve cell from *Astacus* which shows the typical intracellular axone, but this condition is not referred to in the text. Owsiannikow ('00) reported two types of neurofibrillae in *Astacus* cells, the finer fibers forming a network about the nucleus, the coarser fibers being peripherally situated and continuous

with those of the axone. Prentiss ('03) studied the neurofibrillae of the leech and crayfish. "My preparations of *Astacus* showed no trace of fine fibrillae about the nucleus. . . . The fibrillae appear relatively large and form a few large meshes in the peripheral region of the cell." Dahlgren and Kepner ('08) refer to the "implantation cone that reaches far into the cell" in the lobster, and make a general statement with reference to this condition in arthropods, but give no detailed description.

Poluszynski ('11) studied the ganglion cells of *Astacus*, *Squilla*, and *Homarus* with the Kopsch and Sjöval methods, using Golgi's arsenious acid technic as a control. In opposition to Holmgren he differentiated between the intracellular prolongations of the capsular cells and the 'Golgi-Kopsch apparatus,' and concluded that in arthropods generally the latter appears as a series of isolated threads and granules, and not as a network. Since his results are based primarily upon the osmic acid preparations, it is by no means clear that his internal reticular apparatus may not include other constituents of the cytoplasm.

R. Monti ('14 and '15) has concluded that Poluszynski did not see the true form of the Golgi apparatus. She says (translation):

Poluszynski, using the methods of Golgi and Kopsch, did not find the reticular apparatus; he observed in the nerve cell only short rods sometimes curved or distinct granules scattered throughout the body of the nerve cell. He maintains that this formation of granules or short rods is homologous with the Golgi reticular apparatus. In support of the results of his pupil, Nusbaum concludes that the Golgi apparatus presents diverse forms, as nets, filaments, bacteriform granules, circles, etc.

In further discussion, she says that—

When the reaction is good a figure may be recognized that corresponds to that given by Poluszynski, but upon following the reaction with the greatest care, a figure of extreme fineness is obtained that demonstrates the delicate formation of the structure to be comparable with the Golgi internal reticular apparatus.

With a superb technic at her command, Monti studied the mitochondria and Golgi apparatus in ganglion cells of *Astacus*

and Homarus, comparing them with corresponding elements in insect and mammalian ganglion cells. Monti's figures are the only ones which give Golgi apparatus pictures of arthropod cells which resemble those of other forms, and we may assume that the preparations of other workers were incomplete or unreliable so far as this cytoplasmic structure is concerned. It seems that in these forms the apparatus is very delicate and that there is less tendency toward reticular formation than in vertebrate nerve cells. In addition to the diffuse Golgi apparatus, Monti discovered in the small ganglion cells of young Crustacea prepared by the Golgi method, a minute tightly wound skein of threads at one side of the nucleus which is directly comparable in appearance and position with the 'centrophormien' of Ballowitz or the Holmgren 'canals' as they appear in non-nervous cells.

Monti reaches the conclusion that in the invertebrates a portion of the 'chondrioma,' i.e., the mitochondrial granulation as a whole, becomes the Golgi apparatus and the remainder disappears. This conclusion is based merely on the superficial resemblance between the thick Golgi (arsenious acid-silver) preparations and thin sections stained to show mitochondria. Yet the lack of evidence for such a deduction should not detract from the great value of her morphological contributions. Unfortunately for the present purpose, she did not obtain any satisfactory preparations of the large nerve cells of either *Astacus* or *Homarus*, so her figures cannot be compared directly with those presented in this paper.

Tello ('14) describes a cell in the electric lobe of the brain of the torpedo in which there is in Cajal preparations an intracellular axone almost identical in appearance with that of the crayfish. His text-figure 19 would serve as a figure of the crayfish nerve cell were it not for the presence of a dendrite and of a fibrillar capsule about the nucleus. Because of the closeness of this similarity I give the translation of a part of his description:

As may be seen in figures 19 and 20, the nerve cells of the lobe mentioned (bulb of ray and torpedo) possess a very complicated reticulation. Immediately beneath the membrane lies a dense layer of rather coarse primary filaments arranged in bundles parallel and plexiform,

which to all appearances, without seemingly leaving the region, pass to one dendrite or another. Further, there is a submarginal delicate concentric zone that colors less intensely with silver, in which in a good preparation there may be discovered a complex network of fine filaments, pale, rather thick and circumscribed polygonal meshes. The comparison of these preparations with those obtained by Nissl's method shows that the zone is principally of chromatic granules. This has been well studied and described by Studnička. Immediately following, a fibrillar zone is evident, consisting of dense bundles of unequal thickness, dense, concentric, extending through a great part of the protoplasm. The fibrillar layer is almost always discontinuous; often its bundles double and pass to a deeper layer of whorled and spiral appearance, previously observed by authors and especially by Studnička. At the level of the zone the granules of Nissl are absent or are limited in number. Yet it is most interesting of all that its fibers unite at an angle of the soma, become very pale and delicate (usually appearing redder and clearer than the remainder of the reticulation) and producing the principal contingent of the axone. A section somewhat tangential reveals the whorls that the bundles of the layer describe, as well as the complications of their deep derivation. For underneath the aforesaid fibrillar mass we find a thick, irregular layer of bundles, disoriented, and constituted principally of a reticulation similar to that of the second zone. And finally around the nucleus we observe an obscure capsule composed of compact primary filaments arranged in a dense network which recalls completely the pattern (*emplazada*) underneath the cellular membrane.

Text figure 19 of Tello's description shows an encircling intracellular axone, and figure 20 shows a whorled condition of fibrils similar to the appearance of figures 5 and 6 of my 1915 paper. Heidenhain ('11) describes a similar arrangement of neurofibrils in certain spinal ganglion cells of the frog.

OBSERVATIONS AND DISCUSSION

Intracellular axone and neurofibrillae

Since Apáthy's work revived the interest in the fibrillar structure of nerve cells there has been much controversy as to the relations of the axone fibrils within the perikaryon. This has centered naturally about the possible function of these fibrillar structures. Are they the conducting substance *par excellence*? Are they morphological expressions of the stresses and strains in the ground-substance indicating the direction

in which the nerve impulse travels, or are they simply supporting structures, as Szüts ('14) has suggested?

Das Stützgerüst der Nervenelemente ist ihre neurofibrilläre Struktur. Es ist in mehreren Fällen gelungen, den innigen Zusammenhang zwischen der Gestalt und der neurofibrillären Struktur der Nervenelemente nachzuweisen. Die Neurofibrillen muss man daher für den Träger und die Stütze der Zellgestalt und nicht für spezifische leitende Elemente ansehen.

We are in no position as yet to decide this question. There are two problems, however, of fundamental interest which can be discussed profitably: 1) Do the neurofibrillae exist in the living protoplasm? 2) What is their relation to the ground-substance of the cytoplasm?

The first of these questions has not received the attention it deserves. Most observers have tacitly assumed the preexistence of neurofibrillae in the living protoplasm. The evidence in favor of it is not convincing to say the least. In 1868 Max Schultze, a microscopist of the most exceptional ability, unhampered by any preconceptions, discovered a fibrillar structure in the freshly teased cells of the electric lobe of *Torpedo*. This, as we have seen (p. 43), is as favorable a material for the purpose as the large crayfish ganglion cells. Dahlgren ('15) seems to be the only one who has attempted to confirm this observation. He reported "some trace . . . of neurofibrils" and figures a faint longitudinal striation. This object deserves further study with the use of vital dyes.

M. Heidenhain was impressed with the evidence obtained by Cajal ('07) of the individualistic behavior of neurofibrillae in regenerating nerves. The fact that fibrillar differentiations appear after so many diverse fixations surely indicates that, even if they do not exist as such in life, they represent a peculiar type of organization in the neuroplasm so that their presence in a given cell is strong presumptive evidence of its nervous character.

The controversy in the literature has centered about their morphology in fixed and stained preparations which are assumed to give true pictures of their forms and relations. Two categories

of fibrillae are generally recognized, the coarser fibers or network at the periphery and the more delicate plexus or net about the nucleus. The coarse cytoreticulum figured by Donaggio in the dog is uniform throughout the cell. Such a difference in appearance as compared with Cajal, Bielchowsky, and Bethe preparations is probably due to the peculiarities of the technical procedure.

On general principles it is improbable that any networks pervade living protoplasm. The point of view developed through the progress of colloid chemistry postulates that protoplasm consists of a microscopically homogeneous colloidal ground-substance in which are microscopic colloidal aggregates of granulations, fibrillae, and the like. There are various categories of these, each of which has its own physical and chemical peculiarities. This is not merely speculation. The beautiful cytoreticula that may be seen in the protoplasm of many eggs after acid fixation, sublimate, etc., have been proved to be artefacts by the study of the living protoplasm with modern optical equipment, by the micro-dissection studies and vital staining methods of Kite and Chambers, by the use of less violent fixing agents, and by the convincing centrifuging experiments of Lillie and others. The control of the technical procedure in other tissues by the study of fresh material is of especial importance for the study of the nerve cell, for here the difficulties of observing the uninjured cell are very great. Bensley ('11) found the acinar cell of the pancreas in the guinea-pig particularly favorable for postvital study with and without vital stains. After an extensive series of experiments with fixatives controlled by this means, he found that osmic acid mixtures produced less change than any other. This was especially true of his modification of the Altmann mixture, which he has termed 'A. O. B.' Nerve cells prepared by this method are particularly worth study, although it must be remembered that in such large elements as are considered in this paper it is possible that the acetic acid may often reach the center of the cell before the osmic acid and so produce the picture of an acid reticulum. From a consideration of these facts relative to other cells, I

conclude that it is highly improbable that the neuroplasm of the crayfish nerve cell contains either a fine or a coarse network such as is revealed by the majority of neurofibrillar methods. As Heidenhain ('11) has pointed out, the killing fluids used for silver and gold neurofibrillar methods are poor protoplasmic fixatives. The same may be said of ammonium molybdate, ammonium picrate, nitric acid and aqueous mercuric chloride. In the latter case very different pictures are obtained when one does not treat the material first with iodine and then with pyridine as Donaggio did. The least that we can say is that the existence of a cytoreticulum and the supposed relation of neurofibrillae to a perinuclear net rest upon insecure foundations.

The use of the term reticulum or reticulation in this paper is for convenience and does not commit the author to the opinion that a reticulum exists in living protoplasm.

In his work upon the Crustacea, Retzius ('90), perhaps upon insufficient evidence, reaches the conclusion that the large nerve cells, which are considered in this paper, are motor and the small ones are sensory. Dolley ('13) considers the crayfish nerve cells as divided into two principal groups, the motor and the sensory, the principal differentiating characteristic being the presence of an 'intracellular axone' in the former. On the other hand, Allen ('94) considers that the cells within the ganglionic chain are motor and coordinating and that the sensory cells lie outside the chain. The cells without the 'intracellular axone' are much more numerous than those possessing it.

Let us turn now to my own observations. The general form of the large cell of the crayfish is pear-shaped with the axone leaving the narrow end, the transition into the extracellular axone being gradual rather than abrupt. Not unfrequently the diameter of the axone, about one-fifth to one-fourth the diameter of the cell body, may be slightly greater near the exit from the cell than it is immediately outside. In some instances the axone is almost straight at its exit, while in other cases it shows distinctly short, sharp undulations (fig. 7).

In many nerve cells, especially in vertebrates, the gross origin of the axone is in the axone hillock or implantation cone near the

periphery of the cell, the cone usually being rather sharply differentiated from the cytoplasm immediately in contact by the absence of Nissl substance. Only as a name, other than descriptive, can the term axone hillock be used in connection with the large nerve cell of the arthropod (Dahlgren and Kepner, '08), for the reason that the axone has an intracellular origin and a course almost or quite enveloping the nucleus.

In the large cells of the abdominal ganglia of the crayfish the evident origin of the axone is not a definite implantation cone, but rather it is a band or tract of some width curving about the nucleus and composed of numerous fibrillae originating in all parts of the cytoplasmic mass (figs. 1 to 8, 10, 12, 14 to 21). The main portion of the tract as it curves about the nucleus usually lies at a little distance from it, although in a few cells observed, in a plane of section parallel with the curve of the long axis of the tract, it not only touches the nucleus, but produces a very marked indentation without any break in the nuclear membrane (figs. 2 to 5 and 7). This cannot be an artefact produced by knife pressure, as there is no indication whatever of tearing of the ganglia; and also the indentations do not occur in an isolated section, but rather in several sections of series in different cells. At the place of contact there is no evidence of continuity of the tract with the nucleus. In some of the cells the compact encircling portion of the intracellular axone is very broad and trough-shaped or cup-like with the nucleus situated in the depression of the trough. Figures 20 and 21 show extreme widening; these sections are consecutive, showing one limb of the cut tract on one side of the nucleus and the other limb on the opposite side. Other sections in the series make it evident that the two limbs as shown are parts of the one greatly broadened tract. Figure 10 indicates a similar condition in another cell, while figures 17 to 19 show a sharp angle between the two limbs. Only three cells of the large numbers observed indicated such an extreme widening of the compact portion of the tract. Possibly sections of some of the cells in planes other than those followed might have made such a widening evident.

The fibrillae of the intracellular axone in its more compact portions are more or less sinuous in their general course, which is spiral. The spiral arrangement may be observed in longitudinal sections and also in cross-sections of the tract. In some cross-sections observed, 4μ in thickness, this is shown most beautifully by focusing on different planes. As the objective is raised or lowered bringing different planes into view, there is a very striking appearance of a corkscrew movement on the part of the sections of the fibrillae.

In the gold-toned Cajal preparations and also in the Kopsch preparations diverging fibrillae may be traced out from the compact intracellular axone into the cytoplasm for a short distance. Cross-sections of the tract, and also longitudinal sections, may show such fibrillae coming from the cytoplasm throughout the entire length of the intracellular axone. The greater abundance of the diffusely distributed fibrillae, however is to be found in the basal part of the cell and in the portion of the cell on the side opposite the compact part of the axone.

One cell stained intravital with 0.01 per cent thionin showed a fibrillar feltwork throughout the entire cytoplasmic mass, having the appearance of radiating from a point near the nucleus. The apparent reticulation was denser, of more numerous and finer meshes, in the portion of the cell distal to the axone exit. Some other cells showed a more equable distribution of the feltwork. Many of the cells stained in this way are very small and the fibrillae extremely delicate. The latest attempts at intravital staining with 0.02 per cent thionin in normal salt solution indicate the presence of delicate fibrillae in the same position in the cytoplasm and with the same relation to the nucleus as is so distinctly shown in the fixed and stained specimens. Two and one half cubic centimeters of the thionin solution were injected into the pericardial sinus, with some loss, this being followed by two injections of a like quantity, thirty minutes intervening between two consecutive injections. Twenty minutes after the third injection the crayfish was decapitated and the cord removed into the thionin solution. Some cells were soon found, within twenty minutes, showing faint

lines in the cytoplasm. Forty-five minutes after decapitation of the crayfish a cell was observed with delicate stained lines in the intracellular axone. The color was retained for only a relatively short time after discovery of the cell, but long enough for corroboration of the observation by one of my colleagues. Evidently the cytoplasm showing the lines was stained some time before the expiration of the forty-five minutes; how long before there are no means of knowing. Only one of two interpretations can be placed upon the observation; the appearance is due either to death coagulation or it is due to the presence of neurofibrillae in the living cell. Dahlgren ('15) found "some trace of chromophilic bodies to be seen; also of neurofibrils" in living (?) nerve cells in the electric lobe of *Torpedo*.

Sections stained with 0.01 per cent aqueous solution of thionin following fixation in Heidenhain's corrosive sublimate solution show not only the network about the nucleus, but also the fibrillae of the axone; but here the possibility of artefacts produced by the technic intrudes itself.

The structure as indicated by silver impregnation appears very similar to that shown by the thionin staining. Some of my sections prepared by the method Boulé ('07) used for the earthworm give indication of the endocellular reticulum, although not with a degree of distinctness equal to that obtained by the use of thionin. Possibly a somewhat greater difference in intensity between the perinuclear zone and the region toward the periphery of the cell is shown by Boulé's method than by Donaggio's thionin stain. The intracellular axone in the Boulé preparations may be blackened in a portion of its length, but unaffected in the remainder. Where blackened, the compact portion of the band shows no differentiation whatever into fibrillae, but appears as a dense black mass. In some sections the band is blackened in its course from the periphery of the cell body and in the near vicinity of the nucleus shades off into rows of brownish granulations that encircle the nucleus, but, as indicated, not as sharply defined as shown by the thionin stain. The preparations by Boulé's method gave some detail, while those made by Ranson's method were failures as there was only a diffuse browning of the entire cell.

Sections of cells cut in the appropriate plane show the intracellular axone and its position in relation to the nucleus. Preparations by Bensley's A. O. B. method show the origin of the axone to be very widespread throughout the cytoplasm as a diffusely spreading basket of neurofibrillae within which the nucleus lies (figs. 1 to 6, 15, 16). The composite picture produced by a study of various sections, stained with thionin, with silver, and with acid fuchsin, respectively, is almost identical with the one seen in the acid fuchsin preparations.

One notable difference between the origin of the axone in the crayfish and in most vertebrates and in some invertebrates, as leech and earthworm, is the difference in the locality where the fibrillae unite to form the compact axone. In the vertebrate, the fibrillae usually pass from the region about the nucleus to the point of exit at the periphery of the cell, where they form the axone. In the crayfish, on the other hand, the axone is formed as a tract within the cell body, receiving additional fibrillae along its course even to its exit. Another difference is that the perinuclear reticulation in the vertebrate cell seems to be closed, that is, fibrillae form a closed zone about the nucleus, while in the crayfish most of the fibrillae of the perinuclear reticulation, possibly all, have a free origin within the cytoplasm.

While it is generally known that mitochondria are found among the closely crowded fibrillae of the axone hillock or the intracellular axone whereas the Nissl substance is wanting, yet no explanation of the fact has been given.

Nissl substance

Much confusion has arisen concerning the Nissl substance because of the purely morphologic point of view of many investigators (Cowdry, '12, p. 18). This substance is the only constituent of the cytoplasm that has been placed upon a firm chemical basis. Held ('95, '97) was the first to study it from this point of view and to postulate its nucleoprotein character. In 1897 Mackenzie reported the presence of organically bound iron in many of the Nissl bodies by the means of the Macallum microchemical test for iron. This work was greatly extended by Scott

('99), who also demonstrated the origin of the Nissl substance from the chromatin of the nucleus in the neuroblast of the pig. Because of its demonstration side by side with the other recognized elements of the cytoplasm, there is no reason for confusing it with other substances, in the vertebrates at least, and probably also in the arthropods. In no form has its morphology been adequately cleared up, nor can we hope for this until it has been studied by means of a vital dye in a living nerve cell in situ. No such specific dye has yet been found for the Nissl substance or for any other form of chromidial substance. There are no means, therefore, of knowing whether any of the granules seen in fresh cells are 'cytochromatin,' as Heidenhain ('11) has termed it. In certain small spinal ganglion cells of vertebrates (Cowdry, '14), and in the acinar cells of the pancreas (Bensley, '11) the chromidial substance seems homogeneously distributed throughout most of the cytoplasm. The colloidal particles seem to be ultramicroscopic in size in these cells. Their absence from the axone hillock where mitochondria and ground-substance are present between the neurofibrillae is significant in this connection, for it shows there is a definite arrangement of the chromidial substance in the cytoplasm. Its absence from the great axone tract of the large crayfish cells is very striking (cf. figs. 10 to 12). It is possible that in this case, as in other cells where fixing agents produce definite Nissl bodies, the material is present intravital in the form of 'granules,' but of this there is no evidence.

Nissl bodies are figured and described by many authors as they appear in fixed and stained cells, but it is very questionable if they exist as bodies in living nerve cells. Mott ('12), Marinesco ('12), and Cowdry ('14) could not find the bodies in postvital material. Failure attended by efforts to observe them in the living nerve cell of the crayfish. It is only after fixation that they become evident. I teased out individual cells from the abdominal ganglia in normal (0.75 per cent) salt solution, and examined them under low power, 4-mm. objective and no. 4 ocular, and under a 2-mm. Zeiss apochromatic oil-immersion objective and no. 4 ocular. Some of the cells were in their

normal shape, the cover-glass being supported, while others were flattened by pressure upon the cover. In no instance was there any indication whatever of Nissl bodies. The cytoplasm is quite uniform throughout in its appearance, with numerous granules more or less irregular in shape and size and optical appearance, distributed with a marked degree of regularity. Some of the granules are almost at the limit of vision with the 2-mm. oil-immersion objective and no. 4 ocular; others are much coarser, being several times greater in diameter. In shape they vary from almost spherical to an angular outline. Some of the granules, at least, are mitochondria, as may be demonstrated by staining with Janus green. There is no evidence of a grouping of granules into concrete bodies. Intravital staining with methylene blue and with pyronin failed to demonstrate any grouping. Nissl bodies are of such size that they should be observable if they are present in the living cytoplasm, unless such observation is prevented by optical characters. In so far as I have been able to observe, they do not exist in the living nerve cell of the crayfish as formed bodies.

The question involved is not as to the existence of Nissl substance, but as to the morphology of the substance, whether it exists in the condition of dispersed granules or in the form of masses, or homogeneously dispersed through the cytoplasmic ground-substance. The substance exists in living protoplasm but in all probability only in the dispersed condition.

Golgi internal reticular apparatus

The Golgi internal reticular apparatus has been demonstrated in nerve cells from many sources, by certain technic taking on stain and by other technic remaining as clear unstained spaces of various sizes and forms. Misch ('03) reported some vertebrate nerve cells as not showing the presence of the 'Binnennetz.' Perhaps the results obtained by Misch may be attributed to imperfect technic. On the other hand, Cajal ('03) has found the apparatus in every type of nerve cell he has examined, and he believes it to be universally present in all vertebrate cells.

Some workers think that the apparatus is an artefact (cf. Cowdry, '12). It seems to be definite that at least some of the clear spaces appearing in fixed cells from some sources are not artefacts. By the Kopsch osmic-acid method some of the clear canals in a given section may be stained, while others remain unblackened (Cowdry, '12).

In examinations of the living nerve cell of the crayfish I could not distinguish any apparent reticulations of clear spaces in the cytoplasm; the entire mass was seemingly a mixture of granules in a more fluid matrix. Nor did intravital staining with thionin, methylene blue, or with pyronin show their presence. The fact that failure attended the search for the apparatus in the living cell does not give much evidence against its presence. The cell is of such a thickness and it contains so many granules of various sizes that it would be difficult to demonstrate spaces devoid of granules unless the spaces were relatively large. Clear spaces or canals appear in the preparations by the Bensley method, as shown in figures 1 to 6, 8 to 12, 15, 16, and in preparations fixed with osmic acid, Heidenhain's corrosive sublimate, etc., but not quite so clearly in the Carnoy preparations. These unstained spaces are connected with somewhat wider elongated spaces radiating from the path of the intracellular axone. Numbers of attempts were made to demonstrate the apparatus by the Kopsch osmic method, by Veratti's method, and by Cajal's modified uranium nitrate method, these invariably resulting in failure.

If Nissl bodies do not exist as concrete masses of cytoplasmic material in the living cell, then it is very evident that an agglutination is effected by the reagents. As a mechanical result of such action granules might be withdrawn from some portions of the cytoplasm, giving those portions a clear appearance. The shape of the spaces would be determined by mechanical forces. Mathematical regularity would not result, but rather irregularity due to a lack of perfect homogeneity of the cytoplasm. The general appearance of the clear spaces is that of an irregular reticulation. Some of the Nissl bodies tend toward a polygonal outline, while others are somewhat elongated. The

shapes of the bodies and of the spaces are correlated. In the earlier part of my work I was of the opinion that these spaces are the Golgi internal reticular apparatus, but now I believe them to be artefacts.

Poluszynski's work ('11) on the ganglion cells of *Astacus*, *Homarus*, and *Squilla*, chiefly by the Kopsch method, but also by the original method of Golgi as a control, shows the structures stained by this method very variable in appearance. Instead of an 'apparato reticulare' present in most cells, he finds a series of isolated structures darkened by the procedure. He believes this 'granular' form of the Golgi-Kopsch apparatus to be characteristic of arthropods, certainly of Crustacea. His figures of the cells of *Astacus* show bodies of the same order of magnitude as the reticular apparatus of other nerve cells, but he is somewhat doubtful whether they are normal structures. He considers the most typical form of the apparatus to be delicate filaments blackened by the Kopsch method in *Homarus* cells. By this same method I have demonstrated similar filaments in *Cambarus* cells, but I am convinced that they are not the Golgi apparatus, but are fine ramifications of the trophosphonium which anastomose, thus differing, from the reticular apparatus in *Astacus* as described by Poluszynski. The filaments are much more delicate than the clear spaces observed in the same cells after A. O. B. fixation. It may be said also that the Kopsch method is not to be considered specific, since under certain conditions the mitochondria also are well stained by it.

The figures given in the papers by Monti represent elongated threads of various shapes convoluted and frequently anastomosing that in no way can be considered homologous with the clear spaces appearing between the Nissl bodies in my preparations. The threads are more regular in diameter and do not form such a continuous reticulation. Both of the papers tend to corroborate my conclusion that the clear spaces between the Nissl bodies in my preparations are not the Golgi reticular apparatus, but are mechanical artefacts. There can be no question but that the structures observed by Monti in the small nerve cells of *Astacus* and *Homarus* are the Golgi apparatus.

No one has succeeded in obtaining preparations of the large crustacean nerve cells we are here considering which could be interpreted as revealing a typical Golgi apparatus, nor have I had any better success. Whether it is absent or whether the osmotic conditions are such as to make it difficult to obtain complete impregnations in these giant elements remains for further work to decide. Figure 13 is made from a drawing of a cell in a Golgi arsenious acid preparation and represents the only type of positive stain that has been obtained in large nerve cells. It obviously bears little resemblance to the Golgi apparatus as we find it in other nerve cells. These isolated elongated elements are of a greater order of magnitude than those obtained by Poluszynski in Kopsch preparations and may possibly represent greatly swollen mitochondria.

Trophospongium

In some cells in ganglia treated by the Kopsch method very fine blackened bodies, either short or elongate, are evident. Some of them are not larger than mitochondria. Some appear as very delicate rods varying from very short to elongate filaments. These seem to have no relation in position to the clear spaces between the Nissl bodies, but are distributed promiscuously throughout the cytoplasm, and are more numerous in some cells than in others, while in yet other cells none are observed. The more delicate of the filaments are usually found in the interior of the cell and the larger toward the periphery. In some cases those toward the periphery are demonstrably portions of the trophospongium, a more or less complex framework continuous with sheath cells and penetrating into the cytoplasm. It seems evident that the very small blackened filaments in the interior of the cell are fragments of the trophospongium and that the elements of the trophospongial framework are present throughout the mass of the cytoplasm even to the center of the cell, and that they attain such delicacy as to tax microscopic vision. That these filaments are not of the Golgi reticular apparatus is indicated by their extreme delicacy, and especially by their connection with the framework invaginated from the

sheath cells. They indicate a delicacy and an intricacy of structure of the trophospongium more marked even than previously reported (Ross, '15). The finely filamentous structures described by Poluszynski ('11) in *Homarus* may well have been trophospongium stained by the reticular methods, since he did not appreciate the extreme delicacy of this supporting framework.

Mitochondria

"Mitochondria may be provisionally defined as substances which occur in the form of granules, rods and filaments in almost all living cells, which react positively to Janus green and which, by their solubilities and staining reactions resemble phospholipins and, to a lesser extent, albumins" (Cowdry, '16, p. 425).

Altmann added another element to the known complexity of the nerve cell a quarter of a century ago when he observed that in adult nerve cells there are minute bodies now known as mitochondria. Since that date the literature upon mitochondria has become voluminous, although most of the work has been of recent years. Mitochondria are now known to be present in the cells of nearly all tissues of both animals and plants. "They (mitochondria) occur in almost all cells . . . they are as characteristic of cytoplasm as chromatin is of the nucleus" (Cowdry, '16). Mitochondria are readily demonstrable in the crayfish ganglia, in the cell bodies and along the course of the fibers. Such variations in size and shape as are usual in cells from other sources are to be observed here. Some are granular, others short rod-shaped, or of longer rods, straight or slightly bent; granules or rods isolated or grouped or arranged in rows giving a broken line appearance (figs. 9 to 12).

As it was not the purpose of the study to investigate the technic of mitochondria staining, only a few methods were used. A few trials of Regaud's method ('10) were made without satisfactory results, as no mitochondria were made evident. Janus green gave its typical reaction. Bensley's acetic-osmic-bichromate acid-fuchsin method yielded the best results. Although this stain is not permanent, yet it was retained quite distinctly for two and one-half years or longer.

SUMMARY

1. The intracellular origin of the axone of the large nerve cell of the crayfish consists of a large number of neurofibrillae widely distributed in the cytoplasm and almost surrounding the nucleus. The fibrillae converge to form an intracellular axone deep within the cell body, whereas in some invertebrates and in most of the vertebrates the fibrillae converge to an axone hillock near the periphery of the cell.

2. By means of intravital staining some evidence of the probable existence of neurofibrillae in living cytoplasm is obtained.

3. The Golgi internal reticular apparatus was not demonstrated.

4. Nissl bodies were not demonstrable in living cells, but are evident in fixed and stained cells. The bodies are probably artefacts, although there is no reason to doubt the chemical entity of the Nissl (chromidial) substance.

5. Mitochondria are readily demonstrable in the cell bodies and along the course of the fibers.

6. The trophospongium shows connection with the sheath cells, and may be traced as very delicate filaments, in section, penetrating even to the center of some cells.

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EXPLANATION OF FIGURES

All figures are of abdominal ganglion cells of *Cambarus*. Figures 1 to 16 show the distribution of the neurofibrillae and the origin of the intracellular axone. Mitochondria are shown in figures 9 to 12. Photomicrographs and drawings, except figure 14, were made by the author from sections 4μ in thickness, except figure 13, from a section 7μ in thickness. Figure 14 was drawn by Mr. Streedain, to whom acknowledgment is due for valuable suggestions.

ABBREVIATIONS

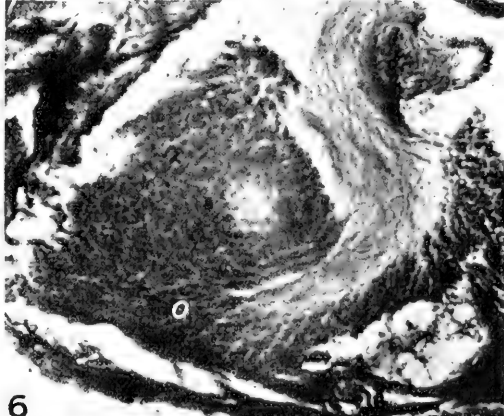
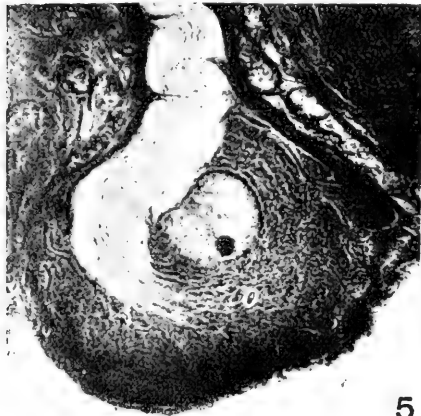
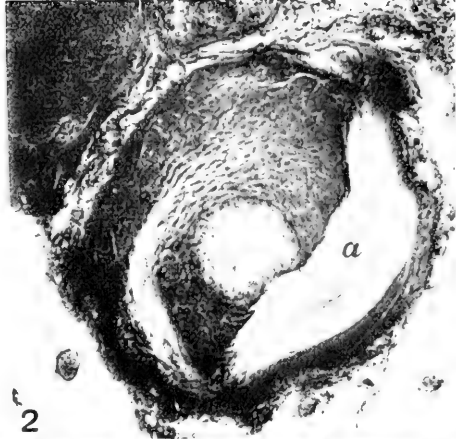
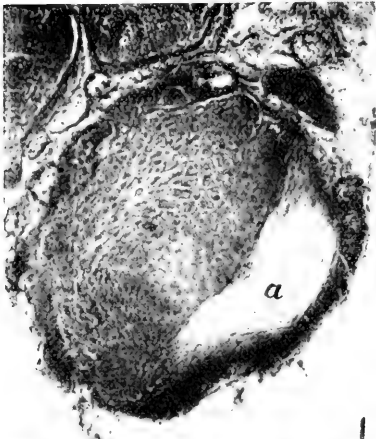
<i>a</i> , intracellular axone	<i>n</i> , nucleus
<i>c</i> , clear spaces, artefacts (?)	<i>o</i> , origin of intracellular axone
<i>m</i> , mitochondria	<i>tr</i> , trophospongium
<i>N</i> , Nissl bodies	<i>un</i> , undulations in intracellular axone

PLATE 1

EXPLANATION OF FIGURES

The sections illustrated in plate 1 are from abdominal ganglion cells prepared by Bensley's A. O. B. method, and were cut approximately parallel with the long axis of the intracellular axone. Sections are 4μ in thickness.

Figures 1 to 4 represent photographs of a series of sections from a single cell, showing the intracellular axone curving about the nucleus and in contact with it in figures 2, 3, and 4, but without organic continuity. Figure 4 shows a lateral bending of the axone fibrils as the plane of the section passes to one side of them at the right of the nucleus. Figure 5 is of a section from another cell, and it likewise shows contact of axone fibrils with the nucleus. Undulations of the intracellular axone are to be observed near the point of exit from the cell body. Figure 6, from yet another cell, shows better than the others the diffuse character of the intracellular axone not far from its exit. Neurofibrillae are evident in all the sections, but most distinctly in figure 6. A reticulation of clear spaces, artefacts (?), is evident in all the figures. Magnification: figures 1 to 4, $\times 539$; figure 5, $\times 576$; figure 6, $\times 563$.



5 6

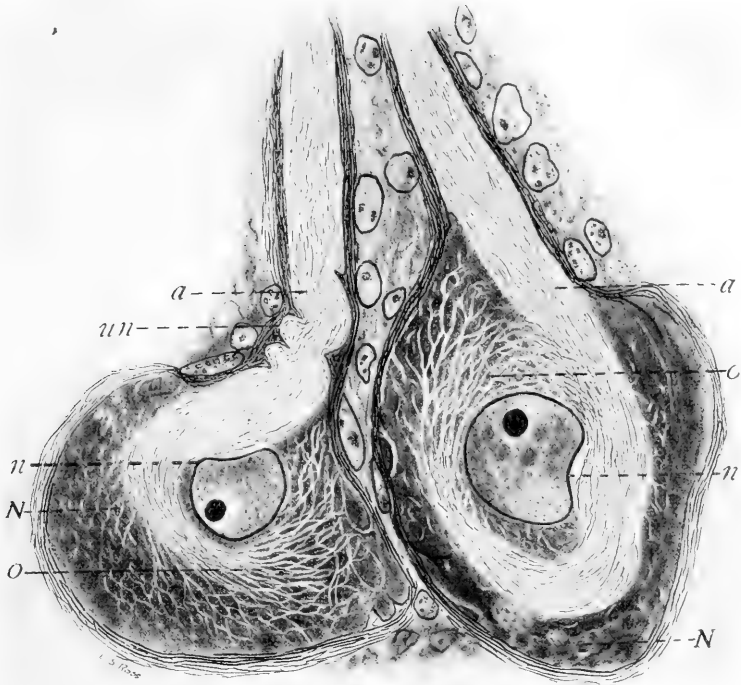
PLATE 2

EXPLANATION OF FIGURES

Figure 7 is diagrammatic to the extent that it was drawn from two different sections in the series, one cell from one section and the other from one three sections farther along in the series. Several sections show the two cells side by side. The plane of the section lies parallel with the intracellular axones and with the curve around the nuclei. Here also, as in figures 2 to 5, plate 1, the axone fibrils are in contact with the nuclei, forming indentations. One cell shows marked undulations in the axone near its exit from the cell body and shows also the neurofibrillae in a position parallel with the undulating surface. The neurofibrillae could not be traced far into the cytoplasm. The clear reticulations show continuity with spaces radiating from the path of the intracellular axone.

Figure 8 is a photograph of a section from another specimen. In this the curve of the intracellular axone is in the opposite direction from that in figure 7. The plane of section lies within one axone for a slightly greater distance than it does in the other, in the one being more nearly through the center of the nucleus.

Both figures are from A. O. B. preparations. Magnifications: figure 7, $\times 562$; figure 8, $\times 750$.



7



8

PLATE 3

EXPLANATION OF FIGURES

Figures 9 to 12 show the clear reticulations, Nissl bodies, mitochondria in the cytoplasm and in the intracellular axones, and small portions of the trophosphonium. Figure 10 shows the compact portion of the somewhat cup-shaped intracellular axon partially encircling the nucleus, a condition observed in but few cells. Figure 12 illustrates a section whose plane is parallel with the curve of the intracellular axon. Only a relatively small part of the axone appears in figure 11. The mitochondria are distributed irregularly throughout the cell body; they appear in large numbers in the intracellular axone, with their arrangement in general parallel with the neurofibrillae. Figure 13 shows bodies that possibly are greatly swollen mitochondria.

Figures 9 to 12 were drawn from A. O. B. preparations, and figure 13 from an arsenious acid silver preparation. Magnifications: figures 9 to 12, $\times 700$; figure 13, $\times 866$.

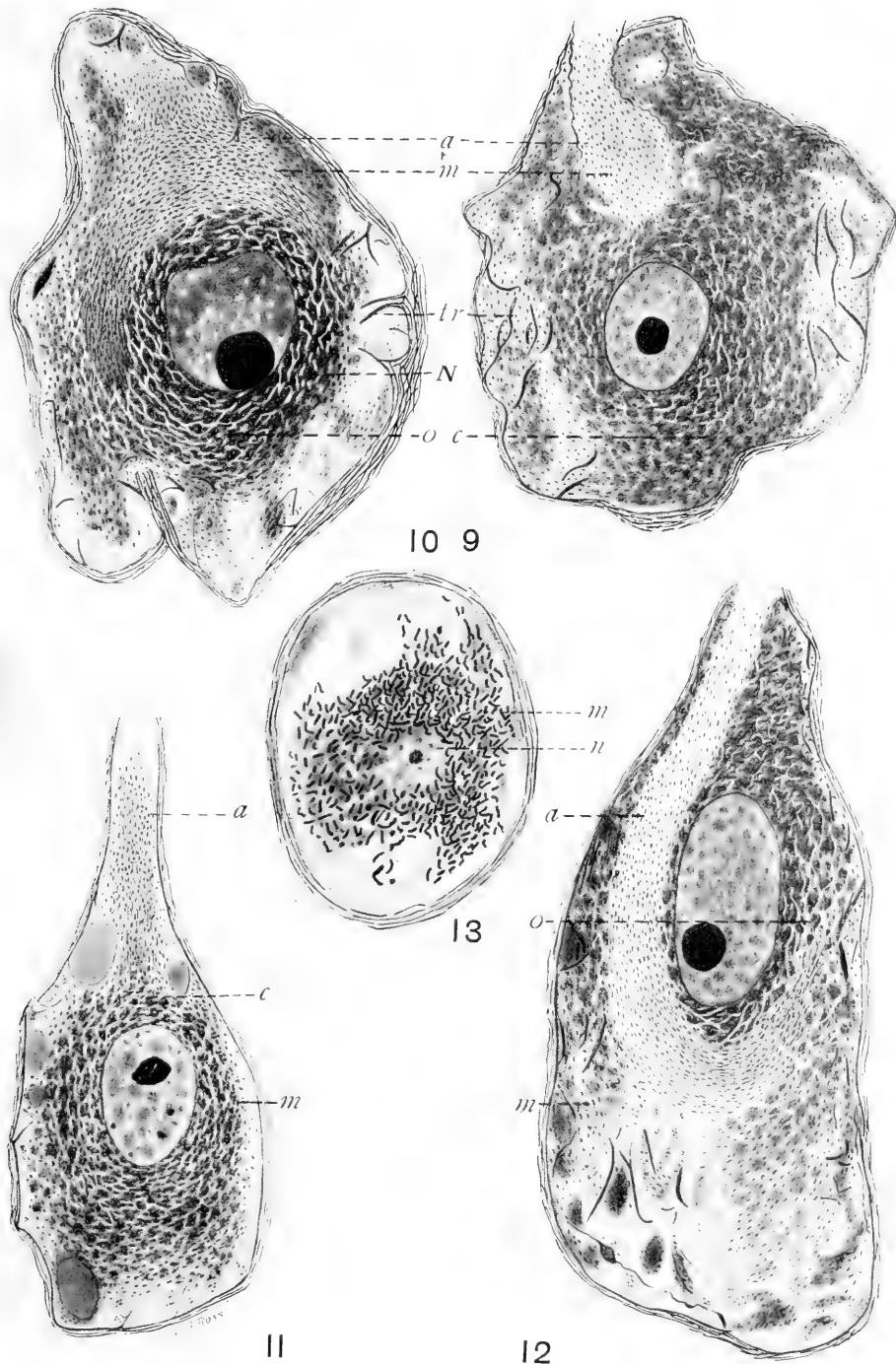
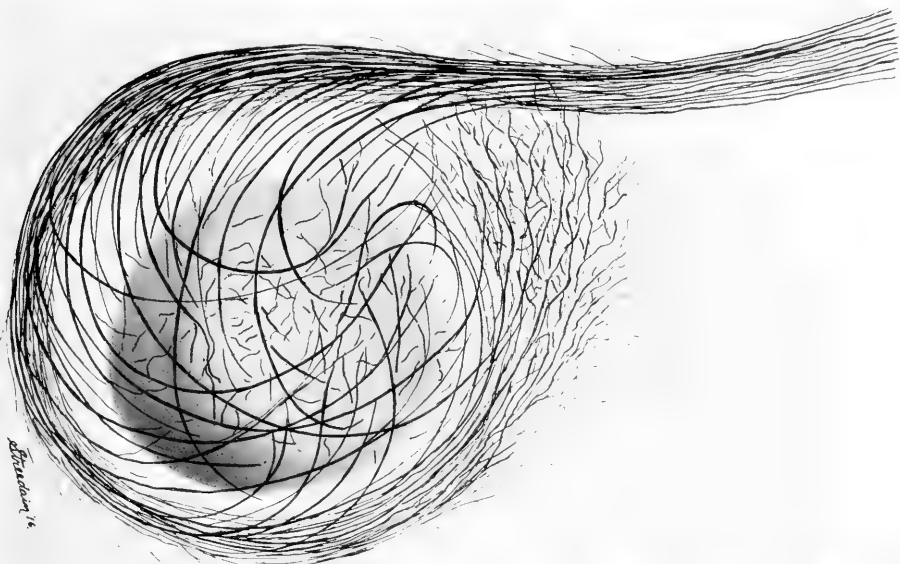


PLATE 4

EXPLANATION OF FIGURES

Figure 14 is diagrammatic and shows the general relationship of position between the nucleus and the intracellular axone. The drawing shows the widely diffuse arrangement of the neurofibrillae as they originate in the cytoplasm and as they converge to form the compact portion of the axone. The nucleus is represented as being free from the axone fibrils, as this is the usual condition. $\times 1200$.

Figures 15 and 16 are photographs from an A. O. B. preparation, both from the same cell. The diffuse origin of the intracellular axone is shown especially well in figure 16. A small portion of the compact portion of the intracellular axone appears in both figures. $\times 600$.



14

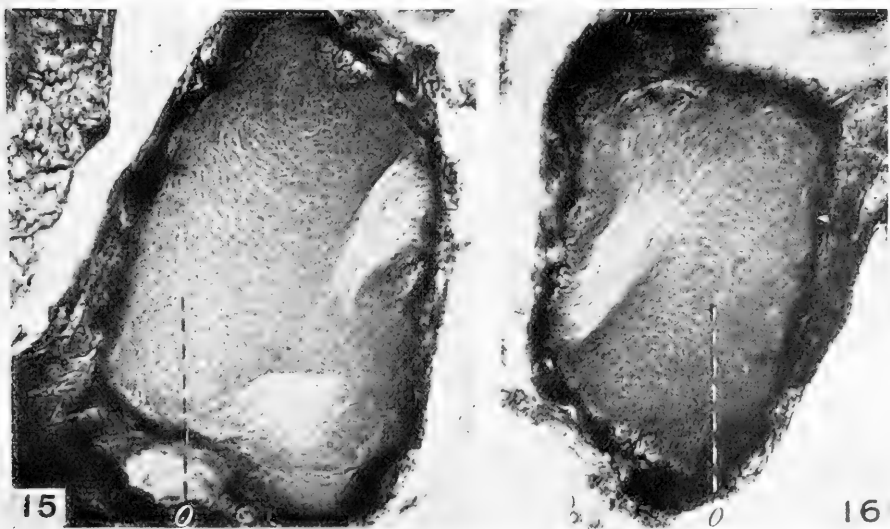
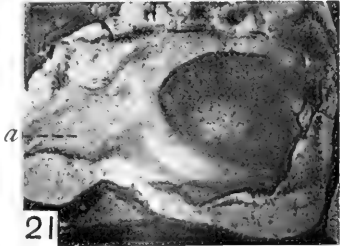
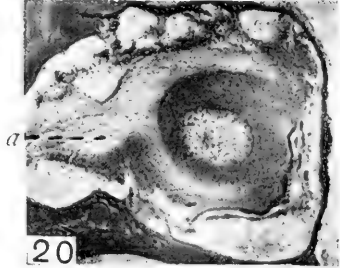
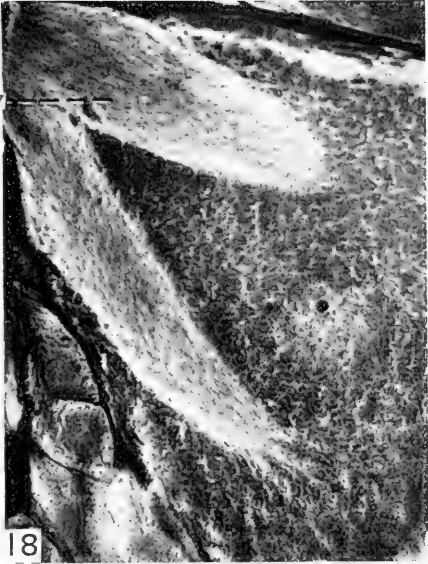


PLATE 5

EXPLANATION OF FIGURES

Sections from two cells are illustrated in this plate; figures 17 to 19 in series from one cell of a Kopsch osmic-acid preparation, and figures 20 and 21 from one cell of an A. O. B. preparation. These are presented to show the wide spreading of the intracellular axone within the cell body that is sometimes found. In figures 17 to 19 the angle between the two limbs appearing in the photograph is acute, while in figures 20 and 21 the limbs join in a wide curve. Magnification: figures 17 to 19, $\times 700$; figures 20 and 21, $\times 327$.



Resumen por la autora, Marion Hines.

Estudios sobre el crecimiento y diferenciación del telencéfalo del hombre.

La fisura hipocámpica.

En la pared media del dehisferio cerebral de los embriones humanos de 16 a 30 mm. de longitud la fisura hipocámpica (Bogenfurche de His) aparece en forma de un surco poco profundo que se extiende desde el bulbo olfatorio hasta el extremo del lóbulo temporal. El hipocampo primordial puede ya reconocerse en los embriones de unos 10 mm. de longitud a consecuencia de su pared más gruesa, matriz más estrecha y un velo marginal más claramente definido que el del área situada lateralmente en inmediata proximidad. Está separado del área epitelial media por el surco limitante del hipocampo. Esta es la primera diferenciación cortical conocida en el hombre. El telencéfalo medio está dividido en el plano medio en una placa terminal y un techo por el ángulo terminal. El área epitelial contigua a la región del plano medio se diferencia en tres áreas características: el septo endimario, el área intercalada y la lámina epitelial.

La fascia dentata procede de la matriz del borde ventral del hipocampo. La fisura primaria de His aparece en embriones de 25mm. coincidiendo con la marcada evaginación del bulbo olfatorio. En las fases tempranas el hemisferio cerebral se dilata mediante crecimiento intrínseco de cada sector particular y por la marcada aceleración del tejido neopial. Se alarga mediante aceleración del crecimiento de la línea media en la lámina terminal y el pliegue ditelencefálico mediante la proyección de arcos de nuevos tejidos que forman los lóbulos frontal, parietal, occipital y temporal. Por consiguiente el crecimiento relativo de las estructuras del plano medio suministra un nuevo método para medir el crecimiento telencefálico.

STUDIES IN THE GROWTH AND DIFFERENTIATION
OF THE TELENCEPHALON IN MAN. THE
FISSURA HIPPOCAMPI

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of Washington, Laboratory of Embryology, Baltimore*

FIFTY-ONE FIGURES

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INTRODUCTION

No question in the history of biological science has gripped the imagination of the student of living matter as much as that of growth. To understand the processes of life, the movement of growth must be studied. In the work to be presented this problem may be approached by singling out movement arrested by death and calling the morphology of its expression stages of growth. It is the interpretation of the morphological and histological changes between these so-called stages out of which the student may build a dynamic conception of growth. The first step in such an analysis is the establishment of landmarks or points from which to measure change. It is the purpose of

this paper to establish landmarks in the growth and differentiation of the telencephalon, such that accurate measurements of the varied components of the developing cerebral hemispheres may be taken. Such a purpose is the outcome of a consideration of the question, long a mooted one, of early telencephalic fissuration. That history is complicated by a group of uncorrelated and seemingly contradictory facts, which bear the names of the most eminent neurologists and embryologists of the latter part of the nineteenth century. The solution of that problem depends upon a more modern technique and a consideration of histological structure.

The reality of certain fissures which appeared in the medial wall of the cerebral hemispheres of the human embryo between the second and the fourth months was under debate from 1868 to 1904. These fissures were variously named, the most important being the arched fissure (or the *Bogenfurche*, the *fissura ammonis*, the *fissura hippocampi*) and the *fissura prima*. The *Bogenfurche* was divided into an anterior and a posterior limb and sometimes radial folds and an arched accessory fissure were added. Are they real or are they artefacts? If they are real, why do they disappear after four and a half months and seem to play no part in the future fissuration of the medial wall? Previous investigators answered them each in his own manner. Those answers have a peculiar bearing upon the present discussion.

HISTORY

Meckel (1815) thought that fissures appeared on the medial wall which later "grew into each other, so that the surface of the brain both inside and outside again becomes smooth." Tiedemann (1816) pictured them, but did not consider them to be transitory; rather, he thought them to represent earlier conditions of permanent sulci. The study of the central nervous system remained fifty years where Tiedemann had left it. Bischoff ('68) reported these fissurations as due entirely to alcoholic fixation. However, in the next year, Ecker reported finding them in the fresh brains of mammalian embryos. Schmidt ('92) referred to these fissures as temporary furrows, but failed

to find them in the sheep, ox, and pig. The maceration artefacts are so great in Marchand's embryos ('91, p. 312) that his description of the hintere Bogenfurche is of little value. But in treating of the vordere Bogenfurche (45-mm. embryo, figs. 1 and 2) he says that the olfactory "bulb is separated from the substantia perforata by a transverse sulcus, which is continued on to the medial surface as the 'vordere Bogenfurche' (incisura prima)." In 1909, although he had recognized the radial folds as artefacts, Marchand nevertheless described a slight indentation (in the fourth month) which extended from the tip of the temporal pole to the region just anterior to the lamina terminalis.

The following year Cunningham ('92) defined the fissures in question as "a series of furrows which radiate in a stellate manner from the fissura arcuata (Bogenfurche) toward the free border of the hemisphere." He believed that "the influence at work in calling the infolding of the cerebral wall into existence appears to be a purely mechanical one, viz., a restraint placed upon the longitudinal growth of the hemisphere; and this being the case it is easy to understand how the number and depth of the fissures will vary with the degree and kind of restraint which is applied" (p. 14). As to their obliteration, he thought that as the cerebral vesicle thickens and the hemisphere elongates, the stellate fissures become detached one by one from the previous arcuata. "In all cases, however, the posterior hippocampal portion is preserved in situ" (p. 16). The anterior part is obliterated at the time of the disappearance of the radial folds. He suggested, as did Anton ('86), that the disappearance of the transitory fissures has some connection with the appearance of the corpus callosum. He cites several cases of radial fissuration in the brains of *Macropus* and *Halamaturus* in which the corpus callosum is rudimentary, and a few instances of congenital absence of the corpus callosum in man. In these brains the radial appearance of fissuration is evident.

In 1898 Hochstetter reported that he could produce the fissuration under discussion by waiting several hours after the death of young fetuses before fixing them. Six years later he challenged His to meet him in Jena. But His could not come. Hochstetter

demonstrated his preparations without a dissenting voice. Those preparations consisted of three embryos, a 13.6 mm., a three months fetus of 50 mm. C. R., and a model of a 19.4 mm. C. R. There was no fissura arcuata in any of these specimens. However, he noted in well-preserved brains, two to four months old, "a slight trough-like invagination of the medial hemisphere wall" (p. 31), but could not identify an arched fissure. Moreover, in speaking of a sixteen-week embryo (p. 33), he says that he can find no trace of the posterior arched fissure, but that the primordium of the pes hippocampi is visible, not as an infolding of the brain wall, but rather as a thickening at that point.

At this meeting Schaper demonstrated the fissureless condition of the medial wall in the two embryos 10.5 cm. and 4.6 cm. Further, he pointed out an insignificant invagination in the region of His' anterior arched fissure in the Hochstetter fetus of 49 mm. The discussion must have attained some warmth or Fick would not have advised that the term fissure be stricken out of embryological terminology. It is difficult to imagine the necessity of calming a morphologist.

Goldstein ('04) described upon the smooth surface of the medial wall "at the point where the anterior arcuate fissure should be sought, a well defined sulcus which extends approximately vertically from the insertion of the olfactory bulb and which His considers the equivalent of his 'Bogenfurche'" (p. 581). This is the fissura prima, and not the true fissura arcuata. For him, the ventricular aspect of the cortex is little disturbed. The broadening and deepening is not an expression of an infolding, but rather a condition of nervous differentiation of the outer level (p. 582). Comparing the posterior arched fissure or the fissura hippocampi with His' figure 86, he says that the fissure is not as deep as that of His; the outer cortex describes only an arched line so that a slight indentation can be seen (p. 586).

In 1901 J. Symington reported at a meeting of the British Association for the Advancement of Science that the frequency and depth of the temporary fissures had been exaggerated, but that they occurred in well-preserved material. Although the arcuate fissure was not a product of fixation, it could have no

morphological significance and was in no way related to the hippocampal fissure. In one brain he was able to trace the hippocampal formation from the region of the temporal pole to that of the developing transverse commissures. He further called attention to the fact, formerly supported by comparative anatomy alone, that the gray and white formation above the corpus callosum in the adult human brain is the remains of a hippocampal formation.

Mall ('03) examined some fifty brains in his collection. He found that those fixed in alcohol or in weak formalin showed fissures on the medial wall, while those fixed in strong formalin showed no such structures. He concludes "according to the experience of Hochstetter, Retzius and myself, the transitory fissures are not found in fresh brains. They are therefore artefacts and of no morphological significance."

G. Elliot Smith ('03, p. 217) says: "Two kinds of so-called 'transitory fissures' have been described in the fetal human brain. There is the group of irregular puckerings of the neopallium, which are found in those fetuses of the 3rd and 4th months in which putrefaction changes have begun; and there is a second group which are found in fetuses of the 5th, 6th and 7th months. It is quite unnecessary to discuss the first group, because their true nature as postmortem wrinklins of the neopallium has been conclusively demonstrated by Hochstetter, and the results of the examination of all known fresh fetuses of the third and fourth months amply confirm the results obtained by Hochstetter's researches."

The heat of the discussion centered around His. But in no instance did he describe the radial folds of Cunningham and the earlier workers. His findings may be compared to those of Retzius ('01), although they share neither the terminology nor the interpretation. In the young embryos (His, '04, fig. 40, embryo C. R. 13.6 mm.) he describes the fissura prima, the anterior and posterior arched fissures and the fissura rhinalis; in later embryos, he adds the fissura arcuata accessoria and the fissura calcarina. The fissura rhinalis and fissura calcarina are permanent and morphologically significant structures. The

fissura prima is the result of the separation of the olfactory lobe, or the anterior smell brain, on the medial side through a medial continuation of the lateral fissura rhinica (p. 76). This fissure does not extend to the lamina terminalis. It remains in the adult as the fissura parolfactoria posterior of the B. N. A. The posterior arched fissura or the fissura hippocampi is a reality because it contains a characteristic structure in no wise connected with postmortem changes. The fissura arcuata accessoria lies above the hippocampal in His' published models. He fails to state whether or not there is any characteristic histological structure present in its depth.

Retzius ('01, p. 92) says that, although the lateral hemisphere wall is smooth, there is a broad sagittally placed furrow or indentation, in the medial wall forming a hillock, which butts into the ventricle. A year later (p. 66) he says the same in regard to the matter.

Such is the history of the transitory fissuration in the human telencephalic vesicle. But what of lower animals? Only two workers have identified structures similar to the findings of Goldstein, Retzius, and His; they are Martin and Grönberg.

Martin ('94) was able to identify the anterior arched fissure in a transverse series of cat embryos, 1.3 cm. in length (p. 224). It appeared later than the fissura chorioidea (0.9 cm.). In the cat, the anterior arched fissure resembles the fissure pictured by His (pl. I, fig. 8, '90). The posterior arched fissure appears as a secondary arched fissure (2.2 cm., p. 226). The union (at 5 cm.) of the anterior and posterior fissures with the 'seitlichen Balkenfurche' (i.e., the sulcus fimbrio-dentatus) he considers to be the future fissure of the corpus callosum (p. 242). This fissure maintains the same relationship to the pallial commissure as the sulcus corporis callosi of the rat (Johnston, '13, fig. 59).

The same sequence of fissuration was followed by Grönberg ('01) in the brain of the hedgehog. The choroid fissure appears in the 11-mm. embryo and the arched fissure in the 15-mm. In figure 54 the inner wall is evidently thickened and forms a slight bulging into the lumen of the ventricle.

This is the first primordium of the hippocampus. The thickening of the wall is limited exactly to the middle part of the groove while the wall retains its original thickness at the transition in the choroid fissure and in the outer mantle, the inner edge forms in cross section a stronger band than the outer (p. 280). The fissura ammonis is not the result of a gradual infolding, but rather is to be considered a secondary formation in the outer layer of the thickened wall (p. 281). If the series is followed through one finds that the fold, if we can give the groove this name, is more accentuated in the posterior portion than in the anterior. A division into two origins, an anterior and a posterior, as His described in the human embryo ('89), I have not been able to confirm (p. 282).

The history of the fissuration on the medial wall of the telencephalon of man, during the second, third, and fourth months, falls naturally into the following subdivisions:

1. Fissuration is an artefact and therefore of no morphological significance.

2. Fissuration is not an artefact. It is accompanied by characteristic histological structure: *a*, without future significance; *b*, with future significance.

If these contradictory statements are true, there is a possible resolution. The solution found rests almost entirely upon a consideration of histological structure and a correlation of development of the tissue in question. To follow the region which lies in the fissura arcuata of His from its earliest differentiation as a tissue distinct from the remainder of the vesicle to its ultimate destiny is the purpose of the present paper. This analysis will give the first point of departure in studying the development of the forebrain as a growing tissue with the hope that at some future time the interrelationship of its various parts may be expressed mathematically.

During the progress of this research I have sought constantly the aid and advice of Dr. G. W. Bartelmez, and depended largely upon the manifold suggestion and the critical judgment of Dr. C. Judson Herrick. Without them it would have been impossible to begin or carry to completion this piece of work. I am happy also to acknowledge the debt I owe Dr. R. R. Bensley, not for aid in this particular problem, but for the scientific training I possess. Further, I wish to acknowledge the use of material

belonging to the Carnegie Institution, Laboratory of Embryology, Baltimore; the kindly interest of the late Dr. Franklin P. Mall and that of Dr. George L. Streeter. Also thanks are due Mr. A. B. Streedain and Miss Marian Manly, of Chicago, for the drawings, to Miss Phelps, of Baltimore, for the microphotographs of the embryos belonging to the Carnegie Collection, and to Mr. Ralph Witherow, for drawings of models of those embryos. And I cannot neglect to acknowledge the debt I owe M. L. Fyffe for an interest, long sustained, in the outcome of this contribution.

MATERIAL AND METHODS

This contribution is based upon a study of human material belonging to the Embryological Collections of the Department of Anatomy, University of Chicago, and of the Carnegie Institution, Laboratory of Embryology, Baltimore. For the elaboration of the technique used in handling human embryos, the Department at Chicago is indebted to Dr. G. W. Bartelmez. The details of this technique are given by Bailey ('16). There is no better human material than that belonging to the Carnegie Laboratory. Doctor Mall was able to secure the cooperation of clinicians so that the preservation of the embryos studied was the best our present technique can secure. Wax models of the brains studied at Chicago were made, while those belonging to the Carnegie were plaster casts poured by Mr. Heard. All these models have been checked many times with either the photographs or the outline projection of the brains in question, so that the writer believes them to be as accurate as our present methods allow.

The embryos studied may be grouped as set forth in table 1.

Besides these embryos the following were examined, although their various olfactory centers were not plotted. In all of them the same areas with the same histological differentiation were found (table 2).

GENERAL MORPHOLOGY

The 11.8-mm. embryo, Mall Collection, 1121 (figs. 7, 8, 9, 11)

So far as the nervous system is concerned, this embryo lies between the 6.9-mm. embryo of His (Br.) and his embryo C. R. (13.6 mm. N. L.). It is a thin-walled tube easily divided into

TABLE 1
Embryos described in this contribution

NUMBER	GREATEST LENGTH	COLLECTION	CONDITION	SOURCE	FIXATION	THICK- NESS
	<i>mm.</i>					μ
1121	11.8	Mall	Good	Abortion	Corrosive sub- limate	40
940	14.0 (10.0 in alc.)	Mall	Excellent	Abortion	Formalin	40
H173	19.1	Chicago	Excellent	Abortion	Formalin- Zenker	10
460	21.0 (20.0 in alc.)	Mall	Excellent	Abortion	Sublimate- acetic	40
H91	27.8 in for- malin.	Chicago	Fair	Abortion	10 per cent formalin	20
H41	32.1 in for- malin.	Chicago	Fair	Abortion	10 per cent formalin	20
H163	39.1	Chicago	Excellent	Operation for fibroids	Formalin- Zenker	20
886	43.0	Mall	Excellent	Operation	10 per cent for- malin and Bowen's fluid	100

the five brain vesicles of v. Baer. The roof plate is thin in the telencephalon and myelencephalon, but not noticeably so in the diencephalon and mesencephalon. The floor plate is beginning to show its characteristic thickenings. The floor of the fourth ventricle is long, broad, and shallow. The lateral recess is not present. There is no evidence of any increase in thickness of its anterior lip. There is an insignificant groove in the floor

of the fourth ventricle, continuous with a deeper groove in the wall of the cord and of the mesencephalon. This is the sulcus limitans fig. 8, (*Sul. lim.*). The part of the mesencephalon which

TABLE 2
Other embryos consulted for this contribution

NUMBER	GREATEST LENGTH	COLLECTION	PLANE OF SECTION	THICKNESS	CONDITION
	<i>mm.</i>			μ	
163	9.0	Mall	Transverse	20	Excellent
H566	11.6	Chicago	Transverse	15	Excellent
H 4	13.7	Chicago	Transverse	10	Poor
H398	14.5	Chicago	Horizontal	25	Excellent
719	15.0	Mall	Transverse	40	Fair
H 5	16.0	Chicago	Transverse	15	Poor
H465	16.0	Chicago	Transverse	15	Good
317	16.0	Mall	Coronal	20	Good
406	16.0	Mall	Sagittal	20	Good
492	16.0	Mall	Coronal	40	Excellent
H516	17.0	Chicago	Transverse	15	Good
576	17.0	Mall	Sagittal	15 and 20	Excellent
1390	18.0	Mall	Sagittal	25	Good
432	18.5	Mall	Sagittal	20	Good
431	19.0	Mall	Sagittal	20	Good
H202	20.2	Chicago	Horizontal	25	Fair
H 19	20.6	Chicago	Transverse	20	Fair
840	24.8	Mall	Transverse	50	Good
455	24.0	Mall	Transverse	20	Good
632	24.0	Mall	Sagittal	40	Fair
H 39	25.0	Chicago	Transverse	25	Fair
405	26.0	Mall	Sagittal	40	Good
1008	26.4	Mall	Sagittal	40	Excellent
H 50	29.2	Chicago	Transverse	25	Fair
878	36.0	Mall	Sagittal	100	Good
H 98	38.4	Chicago	Horizontal	25	Fair
448	52.0	Mall	Sagittal		Good
267	59.0	Mall	Sagittal		Good
H 44	60.0	Chicago	Transverse	25	Fair

lies dorsal to this sulcus is thin and expanded. The part which lies ventrally foretells the future thickening of the mesencephalic floor. This ventricular groove passes through the diencephalon and seems to lose itself in the vicinity of the cavity of the optic

evagination (*Rec. op.*). Immediately dorsal to this groove in the diencephalon lies another, here termed sulcus dorsalis (fig. 8, *Sul. dors.*), which to all appearances arises from the sulcus limitans rostral to the meso-diencephalic boundary. Further forward the sulcus limitans and the sulcus dorsalis fade out in the thalamic wall, dorsal and anterior to the optic ventricle.

These two sulci divide the diencephalon into three regions: a large dorsal region, an insignificant central region, here termed the midthalamic region, and a large ventral region, the hypothalamus. The first or epithalamus is bounded rostrally by the velum transversum (fig. 8, *Vel. trans.*), dorsally by a thin ependymal roof (fig. 8, *Dien. r. pl.*), and the epiphyseal evagination (fig. 8, *Ep. ev.*). The floor of the hypothalamus contains the shallow mammillary recess (fig. 8, *Corp. mam.*), the broad infundibular area (fig. 8, *Inf.*), and the bed of the optic chiasma (fig. 8, *F. b. op. ch.*). The infundibulum can be identified as that area to which the anlage of the anterior lobe of the hypophysis clings (fig. 8, *Ant. l. hyp.*).

The midline of the telencephalic roof is only a little lower than the two lateral vaults of the hemispheres. Consequently the foramen interventriculare (fig. 8, *For. int.*) is only a little smaller than the entire cavity of the whole evagination. The only point of constriction lies in the most dorsal part of the di-telencephalic groove, the region of the velum transversum (fig. 8, *Vel. trans.*).

Following the midline structure forward from the velum transversum, it remains membranous as far as the massive lamina terminalis (fig. 8, *Lam. term.*), below which a slight constriction marks the preoptic recess (fig. 8, *Rec. preop.*). Next comes the chiasma ridge (fig. 8, *F. b. op. ch.*) at the di-telencephalic junction. The corpus striatum is barely visible as a slight ventricular eminence in the floor of the interventricular foramen.

The 14-mm. embryo, Mall Collection, 940 (figs. 10, 12, 13, 14)

A difference of only 2.2 mm. between this embryo and no. 1121 has wrought a marked change in the growth of the central

nervous system. The basal plate of the cord has grown much thicker. The pontile flexure is evident, but not as accentuated as His pictured for the 10.6-mm. in his collection; nor is the floor plate of the medulla oblongata as he showed it. The lateral recess has appeared. There is no indication of a cerebellar thickening in the superior lip of that recess. The midbrain is not as prominent a feature of the morphology of this brain, due in part to the acceleration in growth of the diencephalon. The mesencephalon is separated from the rhombencephalon by a marked constriction of the total brain tube, the isthmus. In the 14-mm. embryo the transition from midbrain to the diencephalon is marked off distinctly in the midline by a sudden dip in the vault. The diencephalic portion of the invagination is the posterior limb of the epiphyseal evagination. The basal plate of the midbrain has grown toward the lumen of the ventricles.

The diencephalon is divided into three parts, comparable to those described for the thalamic region of the 11.8-mm. embryo, by the sulcus limitans below and the dorsal sulcus above. The absolute distance between the dorsal sulcus (fig. 14, *Sul. dors.*) and the sulcus limitans (fig. 14, *Sul. lim.*) is greater here than in the younger embryo, but the tissue dorsal to this ridge and that ventral to the sulcus limitans has changed little. Above the dorsal sulcus is a well defined ridge which is still more clearly marked in the 19.1-mm. embryo (fig. 15). The diencephalic roof plate is longer, measuring the distance from the velum transversum (fig. 14, *Vel. trans.*) to the epiphyseal evagination (fig. 14, *Ep. ev.*). The definitive regions of the floor are already outlined. The wall of the recessus mamillaris is not as shallow as that of the 11.8-mm. embryo. The recessus infundibuli is now a definite well in the floor, dipping down into the solid stalk of the posterior lobe of the hypophysis. The bed of the optic chiasma has increased in breadth and thickness. The preoptic and postoptic recesses are actual cavities in the hypothalamic floor. The sulcus limitans ends blindly dorso-caudal to the optic ventricle.

The ventricular communication between the diencephalon and the telencephalon has suffered a tremendous change in its contour. Now, for the first time, the future relationships are determined. The dorsal and terminal boundaries of the foramen interventriculare are no longer an arc of a circle, although they are not as yet roof and terminus meeting each other in an angle as described for H173. Instead of the smooth ventricular ditelencephalic union, that junction is marked by a slight eminence, which is continued forward into the floor of the cerebral hemisphere, the corpus striatum (fig. 14, *Corp. str.*).

The point of entrance of the fila olfactoria (fig. 14, *Fil. olf.*) into the cerebral hemisphere enables us to locate the region of the future olfactory bulb evagination.

The telencephalic vesicle itself extends far beyond the midline, anteriorly and dorsally. Consequently, the great longitudinal fissure now divides the telencephalon into two parts, the cerebral hemispheres, with a small residual telencephalon medium between.

In the 14-mm. embryo the differentiation of the telencephalon medium and adjacent parts of the cerebral hemisphere has advanced to the point where most of the morphologically significant regions can be delineated. In the following paragraphs these will be described and defined.

The telencephalon medium lying between the velum transversum and the preoptic recess (fig. 14) may be divided into dorsal and ventral moieties, the area chorioidea (*A. ch.*) and the lamina terminalis (*Lam. term.*), each of which is again subdivided into two parts. The lamina terminalis ventrally is thick and massive (*pars crassa, p. c.*) and dorsally is a thin epithelium (*pars tenuis, p. t.*). In the course of development the massive part enlarges dorsalward at the expense of the thin part. The area chorioidea consists of two morphologically distinct portions, the tela chorioidea telencephali medii (*Tel. ch. tel. med.*) anteriorly and the paraphyseal arch (*Par. ar.*) posteriorly. In this brain, it is impossible to draw the dividing line between the tela chorioidea telencephali medii and the lamina

terminalis by the angulus terminalis, as it can be done in the telencephalic roof plate of H173 (fig. 16, *Ang. term.*).¹

The peculiar shape of the parapyseal arch is more clearly delineated in the 19.1-mm. embryo (figs. 15,16). It presents the form of a sharply elevated longitudinal ridge which here forms the floor of the great longitudinal fissure between the two cerebral hemispheres and the roof of the interventricular foramen. Posteriorly it is abruptly terminated by the telencephalic limb of the velum transversum (*Vel. trans.*). Anteriorly it merges gradually with the tela chorioidea telencephali medii. The lateral border passes over at a sharp angle into the medial wall of the evaginated cerebral hemisphere (see the cross-sections, figs. 24, 25, 27). That portion of the hemisphere wall which lies contiguous to the parapyseal arch in later stages forms the anterior limb of the lateral choroid plexus (see beyond, p. 87).

The tela chorioidea telencephali medii is of variable length at different ages. It is defined as that portion of the telencephalon medium which lies between the angulus terminalis (fig. 16, *Ang. term.*) and the parapyseal arch. The choroid plexus is never developed in the contiguous portion of the medial wall of the cerebral hemisphere.

The structures which have just been considered all belong in the telencephalon medium. In the adjoining part of the cerebral hemisphere there are two structurally distinct regions, ventrally the massive subcortical olfactory centers of the septum, and dorsally a thin area epithelialis. The area epithelialis in the 14-mm. embryo is structurally uniform throughout its extent, but morphologically it comprises two very distinct regions. The portion ventrally of the angulus terminalis and adjacent to the membranous part of the lamina terminalis is part of the septum

¹ This term is a modification of His' angulus praethalamicus. Judging from figures 44 and 45 ('04), His refers to a sharp change in the direction of the midline. This angle is probably the same as that described in this paper, although it is not the anterior margin of the midthalamus region. He says that the closing plate of the medial hemisphere wall passes over at a sharp angle into the medial thalamic wall. This point may be called the angulus praethalamicus; beside it begins the margin of transition of the thalamic wall into the hemisphere wall, i.e., the margo thalamicus of the latter (p. 66).

(ventro-medial sector of the hemisphere, p. 107) and resembles in form and morphology the septum endymale of the amphibian brain (fig. 14, *Sept. epen.*). Almost the whole of this portion ultimately is thickened by intrinsic differentiation of neuroblasts.

The portion of the area epithelialis lying above the angulus terminalis is permanently membranous. Its ventral border is continuous with the septum endymale, from which at this age it is not structurally distinguishable. Medially it is bounded by the area chorioidea of the telencephalon medium, and dorsally and laterally by the sulcus limitans hippocampi and primordial

TABLE 3

Telencephalic structures in and near the midplane

MIDPLANE STRUCTURES OF THE TLENCEPHALON MEDIUM	CONTIGUOUS AREAS OF THE CEREBRAL HEMISPHERE
Recessus preopticus	Septum
Lamina terminalis	
Pars crassa	Septum
Pars tenuis	Area epithelialis
Angulus terminalis	Septum endymale
Area chorioidea	
Tela chorioidea telencephali medii	Area intercalata
Paraphyseal arch	Lamina epithelialis
Velum transversum	Lamina epithelialis

hippocampus. It extends backward beyond the velum transversum toward the occipital part of the hemisphere (figs. 12 and 14, *A. ep.*), and in later stages it follows the ventral border of the hippocampal formation as far as the tip of the temporal lobe (figs. 17, 18, 20).

In the light of future differentiation, the entire area epithelialis may be further subdivided into: 1) the septum endymale, already referred to; 2) the area intercalata (fig. 14, *A. int.*) lying contiguous with the tela chorioidea telencephali medii (fig. 14, *Tel. ch. tel. med.*) within which choroid plexus is never developed; 3) the lamina epithelialis (*Lam. ep.*) lying opposite to the paraphyseal arch and the di-telencephalic junction and in later stages

extended backward beyond this junction accompanying the differentiation of the hippocampal formation in the temporal lobe (figs. 16, 18). The whole of the lamina epithelialis of these embryos becomes the lamina epithelialis of the adult lateral choroid plexus. The relations above described are expressed in the accompanying table 3.

The morphological changes noted here in comparison with the 11.8-mm. embryo are as follows:

1. The appearance of the pontile flexure and the lateral recess.
2. The marked medial growth of the basal plates in the mesencephalon.
3. Slight advance in the delimitation of hypothalamic structures and more marked increase in the thalamic region lying between the sulcus limitans and the sulcus dorsalis.
4. Change in the angle of the vault above the foramen interventriculare.
5. Marked growth in the telencephalon, i.e., increase in size of the cerebral hemispheres, 1) by dorsal, caudal, and rostral growth, and, 2) by appearance of the corpus striatum as a ridge in the floor of the lateral ventricle.

*The 19.1-mm. embryo, University of Chicago, H 173
(figs. 15 and 16)*

The whole of this brain was not modeled, but from the sections it is evident that the development of both the basal plate and the ganglia of the cord is precocious. The processes of development, already described in the medulla oblongata, have proceeded with a slight shifting of relative morphology only. The floor plate has maintained a progressive thickening. The depth of the lateral recess has increased. The primordium of the cerebellum has appeared in the dorsal lip of the lateral recess. The midbrain has grown medially by a ventricular extension of the basal plate, while the roof plate and the alar plate have increased in thickness.

In this embryo, as in the 14-mm., the changes in the contour of the thalamus and telencephalon are most marked. The two ventricular markings, namely, the sulcus limitans and the dorsal

sulcus, separate the thalamic wall into three parts. The distance between the pronounced dorsal sulcus and the sulcus limitans has increased both absolutely and relatively, so that this middle portion of the thalamus is becoming the most extensive of the three divisions. The anterior extension of the sulcus limitans cannot be traced to the neighborhood of the optic evagination. A new ventricular sulcus has made its appearance. It lies between the medial limb of the corpus striatum and hypothalamus, arising in the recessus preopticus (fig. 16, *Rec. preop.*) and losing itself in the floor of the foramen interventriculare (fig. 16, *For. int.*). Immediately dorso-caudal to this sulcus lies the midregion of the thalamus, which now forms the posterior boundary of the foramen (fig. 15). The dorsal boundary of this midthalamic division can be followed rostrally to the region of the velum transversum.

However, in the younger stage, the 14-mm. embryo (fig. 13), the posterior boundary of the foramen is here formed by the velum transversum, whose diencephalic limb is continuous with the epithalamus, and its floor is formed by the corpus striatum.

In the epithalamus the epiphyseal evagination is more constricted. The roof of the whole is a little thicker. In the hypothalamus the infundibular recess is wide and the posterior lobe is not constricted. The floor and the walls have increased in thickness. In the midline the bed for the optic chiasma has increased in volume by growth, not only antero-posteriorly, but also dorso-ventrally. This region is separated from the massive portion (fig. 16, *P. c.*) of the lamina terminalis by a deep groove, the recess preopticus (fig. 16, *Rec. preop.*).

In the picture of this model (fig. 16) the most striking aspect of the telencephalon medium lying between this recess and the velum transversum is the sharp angle in the midline. This angle, the angulus terminalis (fig. 16, *Ang. term.*), was commented upon in the description of the 14-mm. embryo. Here the anterior boundary of the telencephalic midline suddenly changes its direction and becomes the vaulted roof of the foramen interventriculare. Is this angle a landmark? Can it be used as a fixed point from which to measure change? Further, can it be used as a

fixed point from which to measure the growth of contiguous structures? One thing is certain, it breaks the midline into two divisions, a ventral and a dorsal, whose ultimate outcome in development is characteristic. The ventral limb is thicker than the dorsal limb. It is the lamina terminalis.

In figure 14 (14 mm.) the dorsal part, or pars tenuis (*p.t.*), of the lamina terminalis is much longer than the ventral or massive part (*p.c.*); but in figure 16 (19.1 mm.) these two parts are approximately the same in length. The former subdivision has been called by Johnston ('13) the lamina supraneuroporica. It is a convenient name. The writer would like to use it, but there seems to be no evidence for as complete a separation between the two portions of the terminal plate as Johnston thinks; and, since the last point of closure of the neural tube may lie anywhere, for aught we know, between the recessus preopticus and the velum transversum, it cannot be used to divide the lamina into two morphologically distinct regions. At present the writer thinks there is no fundamental difference in the later stages between the ventral and the dorsal portions of this area, either in development or internal structure.

The dorsal limb of the angulus terminalis (fig. 16, *Ang. term.*) is divided into two regions, the anterior that of the tela chorioidea telencephali medii (fig. 16, *Tel. ch. tel. med.*) and the posterior, that of the paraphyseal arch (fig. 16, *Par. ar.*). Extending laterally into the two ventricles from this arch are the two lateral choroid plexuses. These plexuses are connected across the midline by the vault (here the paraphyseal arch) of the foramen interventriculare (fig. 16), but are not connected posterior to the velum transversum. Here they form a broad shallow fissure in the medial wall, known as the fissura chorioidea. Bailey ('16a) has called this the posterior limb of the plexus and that adjoining the paraphyseal arch, the anterior limb.

Moreover, dorsal to this fissure in the medial wall of the hemisphere and extending more rostrally is a shallow groove. This groove can be followed as an indentation in the medial wall from a region slightly anterior to the angulus terminalis, caudalward to the tip of the temporal pole. This insignificant furrow

is the fissura hippocampi (see groove, fig. 15) and coincides in extent with the stippled area in figure 16.

The greatest change in the di-telencephalon relationship is the ventricular growth in the medial limb of the corpus striatum (figs. 15, 29, and 32). Looking into the cavity of the telencephalic vesicle, several typical markings may be seen. In the latero-ventral sector lies a depression, which divides the nucleus caudatus into a medial and lateral limb. The lateral hillock is a small and insignificant ventricular ridge; the medial, much larger than the lateral, lies in the ventro-medial part of the ventral sector, just lateral to a deep groove which separates the corpus striatum from the septal region. This medial limb of the corpus striatum is most evident from the ventricular aspect of the di-telencephalic union. The septal region which composes the ventral portion of the medial wall ventral to the angulus terminalis and anterior to the lamina terminalis is separated from this portion of the corpus striatum by a deep groove, called by Herrick ('10) in *Amblystoma* and other vertebrates the angulus ventralis (fig. 31, *Ang. ven.*). Further dorsalward in the medial wall of the hemisphere a slight ventricular ridge can be followed, from the base of the beginning olfactory evagination to the caudal pole. This is the hillock made on the ventricular surface by the fissura hippocampi. This ridge is limited ventrally by a sharp turn in the tissue wall, a deep, well-marked sulcus, which will be called the sulcus limitans hippocampi (figs. 16, 29 to 31, *Sul. vent.*). Ventral to this sulcus and caudal to the anterior limb of the parapyseal arch lies the massive choroid invagination. Rostral to this portion of the parapyseal arch, the medial wall ventral to the sulcus limitans hippocampi is very thin epithelial tissue, the area epithelialis (figs. 16, 31, *A. ep.*).

The outer contour of the telencephalic vesicle is undergoing a change, marked not only by growth rostral to the lamina terminalis region, but also now by a seeming swing of the most dorsal portion of the outer di-telencephalic junction caudo-ventrally. This junction lies ventral to the dorsal thalamic sulcus, which divides the midthalamus from its dorsal region. This relationship of the diencephalon to the telencephalon upon the outer

brain surface is foreshadowed in the structure of embryo no. 940.

The morphological changes found in this embryo as compared with the 14-mm. embryo are as follows:

1. Acceleration of the neopallium and appearance of temporal pole.
2. Appearance of the fissura hippocampi.
3. The invagination of the lamina epithelialis, forming the lateral choroid plexus.
4. Increase in length of the lamina terminalis, and the appearance of the angulus terminalis.
5. The acceleration of the medial component of the caudate complex.
6. Great growth of the midthalamic region.

The 20-mm. embryo, Mall Collection, 460 (fig. 17)

The development of the central nervous system as a whole is practically identical with that attained by H 173, the 19.1-mm. embryo (compare figs. 15 and 16 with 17). In this case also only the telencephalon and part of the diencephalon was modeled. Consequently, the morphological description must be confined especially to the growth of the forebrain vesicle.

The relation of structures in the telencephalon medium are the same as those found in H 173. The angulus terminalis is essentially the same. And as noted before, the lamina terminalis is divided into two regions. The diencephalic limb of the velum transversum forms a small elevation which marks the posterior boundary of the foramen interventriculare (fig. 17, *For. int.*). The anterior extremity of the sulcus limitans coincides with the sulcus which divides the corpus striatal complex from the hypothalamus. This rostral end of the limitans was called sulcus Monroi by His. Milhalkovics, quoting Richert, however, includes in the sulcus Monroi not only the rostral end of the sulcus limitans, but also the sulcus separating the medial limb of the striatal complex from the diencephalon.

In the telencephalon itself there is little change. The embryonic fissura hippocampi (lying in the stippled area, fig. 17) extends from the region dorsal to the olfactory bulb (fig. 17, *Olf. bulb*) to the tip of the temporal pole. The greatest difference in the growth between this brain and that of the 19.1-mm. embryo is in the appearance of neopallium on the medial wall of the definitive occipital pole.

In the floor of the lateral ventricle two hillocks lie side by side, separated by a shallow sulcus. The lateral hillock, much larger in this embryo than in H 173, is the lateral limb of the caudate complex. A sulcus separates the medial hillock (figs. 17, 35, and 36, *Cor. str. med.*) from the thalamus. This groove runs up and over the floor of the foramen interventriculare and ends in the recessus preopticus (fig. 17, *Rec. preop.*). Rostral to the medial limb of the caudate complex and making up the ventro-medial portion of the cerebral hemisphere is the septum. This area is limited dorsally by the sulcus limitans hippocampi (fig. 17, *Sul. vent.*) and ventrally by the angulus ventralis (fig. 35, *Ang. vent.*). The angulus ventralis is a shallow groove which lies between the septum and the rostral portion of the caudate nucleus. Its rostral end becomes lost in the evaginating olfactory bulb. The septum itself has increased in thickness by a marked ventricular growth.

There are, then, three morphological differences to be noted in this brain as compared with that of H 173:

1. Increase in the tissue forming the vault of the hemisphere.
2. Growth of the lateral nucleus of the corpus striatum.
3. Dorsal extension of the ventrally thickened portion of the septum.

The 27.8-mm. embryo, University of Chicago, H 91

The foramen interventriculare is no longer elliptical in outline. It is a dorso-ventral slit lying beneath the paraphyseal arch. It is bounded posteriorly by the midthalamus, ventrally by the medial limb of the caudate complex, dorsally by the area chorioidea, and anteriorly by the lamina terminalis. The sulcus

which separates the medial limb of the caudate nucleus from the middle thalamus ends in the recessus preopticus and runs along the di-telencephalic ventricular junction into the floor of the lateral ventricle. The rostral end of the sulcus limitans almost reaches it. The dorsal sulcus which marks the dorsal border of the midthalamus region is almost obliterated. There is, however, a little evidence of it at the rostral end of the diencephalon, where a small ventricular ridge lies on the same level in an antero-posterior plane with the lateral limb of the velum transversum. The roof of the diencephalon has become membranous just caudal to the velum transversum.

The angulus terminalis in the telencephalon medium is more obtuse. The thin roof of the telencephalon anterior to the parapyseal arch has thickened. The dorsal part of the lamina terminalis, the lamina supraneuroporica of Johnston, here called the pars tenuis, has increased in breadth measured from the midplane to the ventricle and in thickness measured dorso-ventrally. This same process has caused an enlargement of the lamina terminalis in all directions (fig. 20, *Lt.*, Bailey, '16a).

The sculpturing within the telencephalic cavity is so modified that the medial and lateral limbs of the caudate nucleus closely resemble each other in the extent of their ventricular expansion. The lateral ridge of the caudate nucleus is as prominent a hillock in the floor of the ventricle as that formed by the medial limb of this nucleus. But the greatest change within the ventricle is due to the increase in thickness of the medial wall, which lies ventral to the sulcus limitans hippocampi and cephalad to the massive portion of the lamina terminalis, the septum. This marked growth of the septal region extends rostrally into the base of the evaginating olfactory bulb. The cavity of the ventricle is almost filled by the lateral choroid plexus. Upon the medial wall the developing hippocampus forms a continuous bulging on the ventricular wall, long and low at the rostral end, sharp and high at the temporal pole. Immediately beneath this ventricular ridge is the sulcus limitans hippocampi. The ridge is due to a slight infolding of the medial wall, the groove on the outer surface being the fissura hippocampi. In this brain there

is no interruption of the fissure. Ventral to this fissure is that of the choroid plexus, whose taeniae lie so near each other that there is no apparent opening.

The caudal pole of the growing hemisphere attached above the telencephalic limit of the di-telencephalic groove is swinging antero-ventrally and carrying with it new tissue, that of the developing neopallium. Consequently the vault is not only increasing in height above the ventral ventricular eminences, but is also increasing in extent simultaneously with the forward and downward growth of the temporal pole.

The 32.1-mm. embryo, University of Chicago, H 41 (figs. 22 and 24, pp. 116 and 117, Bailey, '16 a)

The most striking aspect of the ventricular surface of the thalamus is the deepening of the rostral end of the sulcus limitans and the progressive fading of the sulcus dorsalis. The region which lies between these two grooves occupies the major portion of the ventricular thalamic surface. Dorsal to the sulcus dorsalis lies a ridge in the midline which is accentuated in the model by the irregular trimming of the diencephalic roof plate. This ridge was described by His as containing the stria medullaris and the habenula. It is interesting to note that its anterior end reaches the lateral limb of the velum transversum, while its ventral border (the sulcus dorsalis) gradually fades out anteriorly in the same region. Consequently, the foramen interventriculare is closed posteriorly by the great midthalamus. The hypothalamus remains almost unchanged. Optic fibers are present in the chiasma ridge. The recesses which lie anterior and posterior to the chiasma ridge are deepened. The infundibulum opens into the cavity of the posterior lobe of the hypophysis. The tuber cinereum is thin. The mammillary recess is very shallow.

The rostral end of the sulcus limitans joins the sulcus which separates the hypothalamus from the medial limb of the corpus striatum, and runs over the floor of the foramen interventriculare into the basal portion of the lateral ventricular surface of the diencephalon. This portion of the corpus striatum together with the dorsal part of the lamina terminalis forms the anterior

wall of the foramen. The vault is sealed by the tela chorioidea telencephali medii and the paraphyseal arch. The angulus terminalis has become still more obtuse. The dorsal portion of the lamina terminalis is no longer slender and ependymal; but, rather, growth in all directions so characteristic of this region in its ventral area has involved the whole of the terminal plate from the recessus preopticus to the angulus terminalis. The pars tenuis becomes less in extent as the pars crassa of the lamina terminalis increases in length.

Looking into the ventricle, the lateral and medial limbs of the caudate complex are separated from each other by a shallow sulcus. The lateral component of this complex has increased in growth relatively more than the medial. The ventricular eminence formed by the fissura hippocampi upon the medial wall is broken in the region dorsal to paraphyseal arch, so that there is now an anterior and a posterior segment. The sulcus limitans hippocampi, lying ventral to this eminence, is continuous from the tip of the temporal pole to a region rostral of the lamina terminalis. The septal region lying between this sulcus and the angulus ventralis has grown in length and width, especially in the ventral portion near its point of continuity with the diencephalon.

The outer contour of the cerebral hemisphere is such that superficially the various poles of the adult hemisphere are recognized. The growth which has given this change in the surface is the result of increase in the tissue which is attached medially to the dorsal border of the hippocampus and laterally to the lateral limb of the caudate complex, namely, the neopallium. The most noticeable result of the growth of this tissue has been the swinging of the primitive caudal extremity, posteriorly, ventrally, and anteriorly. Thus the primordium of the temporal lobe is laid down.

The fissura hippocampi is a shallow groove rostral to the lamina terminalis, while in the region above the paraphyseal arch it is barely visible. However, caudal to the velum transversum this fissure is deeper. Moreover, it follows the new direction of growth of the temporal lobe ventrally and anteriorly, so that its shape upon the free surface of the medial wall becomes a semicircle.

The changes which characterize the two embryos last described are:

1. Closure of the choroid fissure.
2. Actual evagination of the olfactory bulb.
3. Plexus formation in the anterior portion of the diencephalic roof plate.
4. Great increase in growth of the midthalamic region.
5. Reversal of relative size of the two limbs of the caudate nucleus.
6. Further thickening of the dorsal portion of the lamina terminalis, i.e., the encroachment of the pars crassa upon the pars tenuis.
7. The incipience of the temporal lobe in the cerebral hemisphere.

8. The fissura hippocampi is not so deep above the area chorioidea in the 27.8 mm. embryo as in the 19.1 mm. or the 20 mm. Anterior to the angulus terminalis and posterior to the velum transversum the fissura resembles that found in the embryos previously described. The formation, however, is continuous throughout. In the 32.1-mm. the fissura hippocampi is very shallow anterior to the velum transversum, posteriorly it is relatively a deep groove.

The 39.1-mm. embryo, University of Chicago, H 163 (fig. 18)

The changes in the medulla oblongata and the midbrain may be seen at a glance. The cerebellum (*Cer.*) appears as a medially growing thickening of the dorsal lip of the lateral recess, but as yet no fusion has taken place in the midline. The floor plate in both the medulla oblongata (*Myel.*) and the midbrain (*Mes.*) has thickened. The floor plate of the latter does not show any of the subdivisions characteristic of the adult mesencephalon. The sulcus limitans (*Sul. lim.*) can be easily followed from the cord through the myelencephalon and mesencephalon into the posterior part of the diencephalon.

The greatest change as compared with H 41 (32.1 mm.) is found in the development of the prosencephalon, especially in the telencephalic portion. In the diencephalon the sulcus divid-

ing the medial limb of the caudate nucleus from the hypothalamus runs over the floor of the foramen and ends in the recessus preopticus (*Rec. preop.*) as described before. This sulcus is joined at its dorsal end by the anterior portion of the sulcus limitans. The hypothalamus is the same as described for H 41. Within its floor are found the recessus mamillaris, the infundibulum, its recessus, the bed of the optic chiasma and the preoptic recess. It is not possible to identify the sulcus upon the medial surface of this thalamus, comparable to the sulcus dorsalis found in the embryos previously described. In the epithalamus the habenula (*Hab.*), the superior commissure (*Hab. com.*), and the epiphysis (*Ep. ev.*) are easily identified. Immediately anterior to the habenula the diencephalic roof plate is non-membranous. Its anterior end, however, extends forward over the paraphysis in the form of membranous pockets, the postvelar tubules of Warren (*P. vel. t.*). These ependymal tubules (Warren, '17, fig. 18, pl. II, p. 125), invaded by vascular connective tissue, seem comparable to the dorsal sac of amphibians and reptiles.

The telencephalon medium joins the rostral limb of the plexus chorioideus ventriculi tertii at the most anterior attachment of the postvelar tubules (fig. 18, *P. vel. t.*). From this point in the telencephalic midline to the preoptic recess the region which shows the greatest growth in length and breadth is the massive portion of the lamina terminalis (fig. 18, *Lam. term.*). Only a small region immediately ventral to the angulus terminalis has remained thin and tenuous. The antero-posterior measurement of the area chorioidea is almost the same as that of the two embryos, 27.8 mm. and 36.1 mm. in length. Of this, the tela chorioidea telencephali medii (fig. 18, *Tel. ch. tel. med.*) occupies its anterior half and the paraphyseal arch its posterior (fig. 18, *Par. p.*). The pouch itself extends over the area chorioidea a real evagination of midline tissue continuous with the velum transversum. In the series this is the only embryo which has the typical circular constriction of the stem of the paraphysis. Although the figure delineates but one sac, there are two small lateral pouches which open directly into the central evagination. These relationships are similar to the one which Warren ('17 fig. 14, p. 121) has described for a 25-mm. human embryo.

In the medial wall contiguous to these structures lie the typical subcortical tissues. The most cephalad of these, the septum (fig. 18, *Sept.*), bulges into the cavity of the lateral ventricle. Dorsal to the septum, lying between the sulcus limitans hippocampi and the pars tenuis of the lamina terminalis, is the thin septum ependymale (fig. 18, *Sept. epen.*). Morphologically it differs in no respect, except in position, from the area intercalata (fig. 18, *A. int.*) adjoining the tela chorioidea telencephali medii. The anterior limbs of the lateral choroid plexuses are continuous with the paraphyseal arch a short distance cephalad to the anterior limb of the paraphysis.

The foramen interventriculare is barely visible from the medial surface. The growth of the midthalamic region, forward and medialward, has restricted its caudal boundary. Its floor is filled with the medial limb of the caudate nucleus. Its anterior boundary is limited by the dorsal region of the terminal plate. Its vault contains the paraphysis and the tela chorioidea telencephali medii. Hence the boundaries of the foramen have not changed, although its diameter is narrower than that found in H 41. The surrounding structures have increased in size, growing toward the center of the foramen in all directions.

Looking down toward the ventricular floor in the rostral pole of the hemisphere, at the level of the lamina terminalis, the anterior and the medial limbs of the caudate nucleus are seen. The medial nucleus increases in width gradually as far as the level of the anterior limb of the paraphyseal arch. From that point posteriorly it becomes narrower. The lateral nucleus of this complex springs from the side wall at the level of the root of the olfactory bulb. It attains its greatest width at the level of the dorsal portion of the lamina terminalis. Caudally it terminates in the region of the posterior extremity of the hippocampus. The angulus ventralis lies between the medial limb of the caudate complex and the lateral extension of the septal region. It extends from the base of the olfactory evagination into the floor of the foramen interventriculare. The two ventral sectors of the hemisphere wall have grown in length, so that the telencephalic evagination projects beyond the lamina terminalis much farther

than in the stage previously described. On the medial wall, the hippocampus does not bulge into the hemisphere except posterior to the region of the tela chorioidea telencephali medii. However the sulcus limitans hippocampi lying ventral to the hippocampus extends from the tip of the temporal pole over the foramen and becomes lost just caudal to the olfactory bulb evagination. The original caudal pocket of the ventricle has swung ventro-anteriorly and carried with it the lateral complex of the nucleus caudatus. The neopallial arch which bridges the lateral division of the corpus striatum and the medial olfactory area is much higher than before. The plexus chorioideus ventriculi lateralis almost fills the cavity. The chorioidal fissure is filled with mesenchyme and blood vessels.

The outer contour of the telencephalon begins to resemble that of the adult. In the center of the lateral surface an area of retarded growth is present, the future island of Reil. Dorsal, caudal, caudo-ventral, and rostral to this point of slow growth, the telencephalon swings out, growing as it were between its points of attachment to the diencephalon. Upon the medial surface the line of separation of the cerebral hemisphere from the evaginating olfactory bulb is carried up on the wall as a small indentation, which terminates rostral to the lamina terminalis. This is the fissura prima of His. Caudal to the velum transversum the fissura hippocampi can be traced to the tip of the temporal pole. Rostral to this point this fissura no longer can be identified on the medial surface of the hemisphere. The changes which have taken place in growth between the two embryos H 41 (32.1 mm.) and H 163 (39.1 mm.) are as follows:

1. Marked evagination of the olfactory bulb, which accentuates the fissura prima of His.

2. Great growth of the lamina terminalis, together with a lateral extension of the septal region into the ventricle.

3. The fissura hippocampi is restricted to the medial wall caudal to the velum transversum.

4. Constriction of the foramen Monroi by the growth of the midthalamic region.

5. No sulcus dorsalis thalami can be identified upon the ventricular surface.

6. Appearance of the island of Reil.

7. Great growth of the neopallium.

The 43-mm. embryo, Mall Collection, 886 (figs. 19 and 20)

This embryo measured 39.9 mm. greatest length in alcohol, only 0.8 mm. longer than H 163. It is not strange that the forebrains of these two embryos are very similar. The development of the cord and the medulla oblongata is the same. The cerebellum appears as a medial outgrowth from the dorsal lip of the lateral recess. The floor plate is very thick. The midbrain is divided into alar and basal plates by the sulcus limitans. The basal plate is increasing in depth. There is no hint of a division into colliculi upon the roof of the mesencephalon. The ventricular markings in the thalamus are the same. The sulcus limitans ends blindly in the hypothalamus. It is joined for a short distance by the sulcus which delimits the ventral boundary of the midthalamus, the sulcus Monroi. Dorsal to this sulcus is the great midthalamic mass. Here as in the 39.1 mm. embryo there is no visible separation between this part of the thalamus and the epithalamus. The epithalamus contains the characteristic structures, the epiphyseal evagination, the habenula, the habenular commissure, and the choroid plexus of the third ventricle. The hypothalamus contains the corpus mamillare, the thin tuber cinereum, the posterior lobe of the hypophysis, the recessus infundibuli. The last recess is deeper in this embryo than in H 163 (39.1 mm.). The recess preopticus lying between the chiasma ridge and the inferior limb of the lamina terminalis is longer and more shallow than that of the 39.1-mm. embryo.

The structures of the telencephalon medium are similar in relation and development to those found in the 39.1-mm. embryo. The lamina terminalis is slightly longer, but not broader. The angulus terminalis is not as well delineated. The length of the area chorioidea is practically the same as that of H 163. Its anterior division, the tela chorioidea telencephali medii is like the 39.1-mm. but its posterior, the parapyseal arch, is not the same.

Taking for its anterior limit that part of the midline where the two lateral choroid plexuses are continuous over the telencephalic roof and the velum transversum as its posterior boundary, its length is approximately that of the 39.1 mm. However, it was impossible to identify a dorsal outpouching in the region of the paraphyseal arch. This may be due in part to the fact that the embryo was sectioned at 100μ , coronal to the cerebral hemisphere. A thinner section in the transverse plane is more advantageous for the study of this region. In the 39.1-mm. this structure was present for about 175μ .

The septum continues its growth into the ventricle. The area of the septum ependymale measures approximately the same and cannot be distinguished morphologically from the area intercalata. The extent of the lamina epithelialis resembles that of the 39.1-mm. embryo.

Within the telencephalon the ventricular ridges have the same relationship to each other as that described for the 39.1-mm. embryo, H 163. The lateral limb of the caudate nucleus is large and ends in the ventromedial horn of the lateral ventricle. The medial limb of the same complex terminates at the base of the olfactory bulb. The angulus ventralis separates this medial limb from the septal region. The hippocampus forms a rounded hillock upon the medial wall which tends to flatten out anterior to the angulus terminalis. The sulcus limitans hippocampi lies beneath it, extending from the tip of the temporal pole to a region slightly anterior to the rostral limit of the fascia dentata. The fissure of the choroid plexus is completely closed. The vault of the hemisphere is higher in the region of the paraphysis than in the 39.1-mm. The anterior and ventro-posterior extension is not greater than that of the last brain described.

The outer contour is not materially changed. The island of Reil is visible on the lateral surface, so that the hemisphere may be divided into various regions indicative of its future lobulation. The olfactory bulb is large and its cavity opens into the ventricle. Along its medial boundary of constriction, extending dorsally anterior to the lamina terminalis lies the fissura prima of His. Posterior to the terminal plate, the fissura hippocampi can be

followed from the region of the paraphysis to the end of the hippocampal formation.

The most marked difference between this embryo and H 163 (39.1 mm.) is the relative growth of different portions of the telencephalon medium. They are as follows:

1. Increase in length of the lamina terminalis.
2. No discernible paraphyseal arch.
3. Increase in the height of the neopallial vault, especially in the central region of the hemisphere.

In the first two brains described, no. 1121 (11.8 mm.) and no. 940 (14 mm.), no fissure or sulcus on the medial wall was visible. But in the description of nos. H 173 and 460, 19.1 mm. and 29 mm., respectively, a shallow groove extended on the medial wall of the hemisphere from the region of the future olfactory bulb evagination to the most caudal and ventral extremity of the lateral area of di-telencephalic fusion. This fissure was also noted in much the same position in H 91 and H 41 (27.8 and 31.1 mm.), although its depth was not as great rostral to the terminal plate. Immediately dorsal to the paraphysis this groove is very shallow, the wall being almost smooth. But caudal to the paraphysis the fissure follows the curved contour of the temporal lobe of the hemisphere, lying in the medial wall, describing an arc of a circle. In the oldest brains here considered (39.1 mm. and 43 mm.), the rostral division of the medial wall is very smooth. However, just posterior to the region of the paraphyseal arch lies the rostral end of an indentation, the fissura hippocampi. This fissure may be traced as a shallow depression in the hippocampus to the caudal end of the primitive temporal pole.

Besides this fissure another was described, the fissura prima of His. It is found only in the older embryos of the series (32.1 mm., 39.1 mm., and 43 mm.), or in those embryos in which the olfactory evagination is prominent. It seems to delimit the tissue basal to the attachment of the olfactory bulb from the cortex dorsal to its point of evagination. It is the former groove or fissure hippocampi which has claimed the writer's consideration for a number of years. And you, the reader, are you ques-

tioning its reality? You may well do so, when its history is remembered. Certain it is that the question of fixation was adequately controlled. The embryos studied were carefully preserved, their further handling as good as our present technique allows, and yet such treatment did not banish from the medial wall of the early telencephalon the fissura arcuata of His (the fissura hippocampi of others). Nevertheless the description of this fissure seems to indicate that its preterminal limb is transient. If this groove is real, there must be some explanation, first for its appearance and second for its partial obliteration. Further, if it is real, a histological differentiation would be expected and processes of growth differences may account, in part at least, for its appearance and later its disappearance in the prevelar region. It is not a question of fixation. A very meager experience with this material gives an unerring criterion to the investigator for the determination of artefacts. The explanation is to be found, rather, in the development not only of the tissue involved in the fissure itself, but also in the histological differentiation of tissue in more remote parts of the developing hemisphere wall.

HISTOLOGICAL STRUCTURE

The 11.8-mm. embryo, Mall Collection, 1121 (figs. 21 to 25)

The telencephalic vesicle of the brain of this embryo is little more than a single evagination, which has expanded slightly beyond its initial attachment to the diencephalon. The lateral expansion of this vesicle is greater in the region midway between its anterior and posterior poles. The midline extending from the region of the velum transversum rostrally is interrupted by an infinitesimal elevation, the paraphyseal arch. Figures 21 to 25 are line drawings of sections of the vesicle cut at right angles to the chord joining the velum transversum and the preoptic recess. The levels are indicated in figures 8 and 11. The midline, beginning in the region of the bed of the optic chiasma, may be divided morphologically into the following regions (figs. 7 and 8 and p. 85 supra):

Region of the optic chiasma (fig. 21, *F. b. op. ch.*).

The lamina terminalis pars crassa (fig. 22, *Lam. term.*).

The pars tenuis of the lamina terminalis and the septum ependymale (fig. 23, *Sept. epen.*).

Region of the parapyseal arch (figs. 24 and 25, *Tel. r. pl.*).

Velum transversum (figs. 7 and 8, *Vel. trans.*).

In the region of the bed of the optic chiasma (fig. 21, *F. b. op. ch.*) the evaginating vesicles form two arcs of a circle, their intersection being marked by a small depression in the midline. Following this arc of the telencephalic brain wall around to its point of union with the diencephalon, it appears fairly uniform in histological structure. In figure 22 the vault of the brain wall is more extensive. Within its outer margin a barely discernible clear zone appears, the Randschicht of His, or the marginal velum (*Man. l.*). This zone is more clearly defined a few millimeters (measured on the figure itself) lateral of the midline. The wall at this point shows a very slight local thickening. In figure 23 the lateral expanse of the vesicle is greater and the marginal velum more sharply defined. The union of the two vesicles is made by a thin epithelial plate of cells. In this figure, approximately 6 mm. on either side of this thin line, no mantle zone can be identified in the brain wall (figs. 9 and 11, *Sept. epen.*). But beyond this region, for 10 mm. on either side, the marginal velum reaches its maximum definition.

Figure 24 is a section through the caudal portion of the telencephalic roof plate, at the level of what appears to be the parapyseal arch (figs. 8 and 24, *Tel. r. pl.*). This small arch in the midline is one feature of a remarkable histological differentiation in the telencephalon. Joining the lateral limbs of this arch is a slender, homogeneous epithelial tissue. Immediately lateral to this tissue is an area in which the marginal velum is clearer and wider than any other portion of the vesicle. Again lateral to this area is a region of the brain wall which forms the telencephalic evagination (*Tel. evag.*) or incipient cerebral hemisphere, extending backward to the point of junction with the thalamus at the di-telencephalic groove. Upon the ventricular surface the first and second areas are separated by a shallow sulcus, which will be referred to as the sulcus limitans hippocampi (fig. 24, *Sul. vent.*).

In figure 25 also on the right side reading from the midline lateralward, the following histologically distinct areas are found: 1) the telencephalic roof plate, i.e., the parapyseal arch (*Tel. r. pl.*); 2) region of the future lamina epithelialis (not labeled); 3) an area which will contain the fornix and the fascia dentata (compare the older embryo, fig. 14); 4) separated from the lamina epithelialis by a ventricular sulcus (*Sul. vent.*), the sulcus limitans hippocampi, is the primordium of the future hippocampus (*Prim. hip*). The sulcus limitans hippocampi can be traced from the caudal level of the parapyseal arch (fig. 11, *Reg. par. ar.*) to a region immediately anterior to the most rostral extent of the arch. This ventricular marking delimits areas at this stage of development already histologically distinct. This sulcus, together with the clear marginal velum lateral to it, enables the writer to bound accurately the anlage of the hippocampus. These slight early differentiations are of great importance, because they can be used as landmarks from which to measure the further growth and differentiation in the telencephalon.

The 14-mm. embryo, Mall Collection, 940 (figs. 26 to 28)

The counterpart of the distinct histological structures, dorsally placed in the telencephalon of the 11.8-mm. embryo, are found either in the unevaginated midline little changed or in the medial wall of the hemisphere of the 14-mm. The hemispheres of this brain extend for an appreciable distance anterior to the lamina terminalis and posterior to the velum transversum. In order to facilitate the description of the shifting position of histologically distinct areas, especially anterior to the lamina terminalis, within the developing hemisphere, they may be divided into quadrants (following the morphological terminology of Herrick, '10). In that paper Herrick suggested a subdivision of the amphibian cerebral hemisphere into four fundamental quadrants, viz., dorso-lateral (pyriform lobe); 2) dorso-medial (primordium hippocampi); 3) ventro-lateral (striatum complex); 4) ventro-medial, septal complex. To these in mammals there is added (p. 496) a fifth region, the neopallium. In this embryo (14 mm.) these regions can be recognized save for the confusion of the pyriform,

or dorso-lateral olfactory areas, with the striatal complex, a condition comparable with that of the adult reptile (Crosby, '17). Because of this confusion and the absence of neopallium in the amphibian, the writer prefers the use of sector instead of quadrant. Accordingly, in this brain each hemisphere can be divided into four sectors: dorso-lateral (neopallium), dorso-medial (primordium hippocampi), ventro-lateral (combined striatum, lateral olfactory area and pyriform lobe), and ventro-medial (septum and medial olfactory area). At this particular level the two dorsal sectors are of approximately the same thickness; the wall of the ventro-lateral sector, however, is much thicker than that of the ventro-medial. The dorso-medial sector may be distinguished from the dorso-lateral by a broad white marginal velum within the medial one (fig. 26, *Prim. hip.*). This same layer of the telencephalic wall within the dorso-lateral quadrant contains many neuroblasts (fig. 26, *Neopal.*). The matrix or zona ependymalis of the medial sector is not as broad as that of the lateral. The dorso-medial region is separated from the ventro-medial by a sharp ventricular groove, the sulcus limitans hippocampi of Herrick (fig. 26, *Sul. vent.*). The ventro-medial sector itself is divided into two histologically distinct areas: a dorsal thin layer of tissue whose cells, packed closely together, show no definite arrangement, the septum ependymale (fig. 26, *Sept. epen.*), and a ventral whose thick wall presents an inner dense matrix and an outer zone, containing a few neuroblasts, the septum (fig. 26, *Sept.*). The two ventral sectors are separated from each other by a slight indentation in the ventricular surface the angulus ventralis (compare Herrick, '10, figs. 13 and 14, A. V. with fig. 26, *Ang. vent.*). Lateral to the ventral angle the brain wall bulges into the ventricle, the first evidence of the corpus striatum, specifically, the medial nucleus of the caudate complex. Between this nucleus and the dorso-lateral sector must lie the future lateral olfactory complex and the lateral limb of the caudate nucleus. The four sectors of the hemisphere are then, 1) the dorso-lateral or the neopallium; 2) the dorso-medial or hippocampal primordium; 3) the ventro-medial or the septum and the septum ependymale, and, 4) the ventro-lateral or the

corpus striatum and the lateral olfactory areas. The particular area which is of immediate interest is the dorso-medial sector, the future hippocampus (fig. 26, *Prim. hip.*). The peculiar histological character of this tissue, already noted in the 11.8-mm. embryo but accentuated here, (that is, the clear practically cell-free marginal velum and the slightly thinner matrix) extends from the region just dorsal of the future olfactory evagination, sickle-like, over the foramen interventriculare to the caudal tip of the hemisphere (see the stippled area in figs. 12 and 14).

Passing caudally, the future hippocampus occupies a progressively more dorsal position in the telencephalic wall (figs. 27 and 28). The amount of neopallial tissue present in the caudal portion of the hemispheric evagination is less than that found at its rostral pole. In figure 27, a section through the paraphyseal arch (*Par. ar.*), there lies between the arch and the future hippocampal cortex (*Prim. hip.*) a curved ependymal wall with the concavity directed outward. This tissue, which joins the hippocampus at the sulcus limitans hippocampi (*Sul. vent.*) and the lateral limb of the paraphyseal arch, is the first stage in the development of the sulcus of the lateral choroid plexus (figs. 27 and 28, *Sul. fu. pl. ch. ven. lat.*).

The regions differentiated as histologically distinct areas are as follows: The neopallium, the hippocampus, the corpus striatum, the septum ependymale, the septum, the two divisions of the area chorioidea, the tela chorioidea telencephali medii, and the paraphysis, together with their respective contiguous structures, the area intercalata and the lateral choroid plexus. Besides these, there is the marked ventro-caudal growth of the hippocampus.

The 19.1-mm. embryo, University of Chicago, H 173
(figs. 15, 16, 29 to 32)

The cell arrangement within the telencephalon of this embryo bears little semblance to that found in the 14-mm. embryo, with one exception, the future hippocampus. This region of relatively cell-free marginal velum can be identified on the medial wall from the region of the base of the olfactory bulb evagina-

tion to the tip of the temporal pole (see the stippled area in fig. 16). The other components of the vesicle have shifted their places in the sector formation, but continue to maintain their fundamental relationships. The future hippocampus now occupies the centro-medial area, in levels anterior to the lamina terminalis (figs. 29 and 30, *Prim. hip.*). This tissue swings gradually upward to those levels further caudalward (figs. 31 and 32, *Prim. hip.*). The ventral margin is limited throughout by the sulcus limitans hippocampi (figs. 29 to 32, *Sul. vent.*). The groove which outlines the future hippocampus on its medial surface is the fissura hippocampi (figs. 29 to 32, *Fis. hip.*). Not only is the future hippocampus distinguished by the cell-free outer zone, the marginal velum, but also, by the narrower matrix, and a greater thickness of the brain wall itself. Immediately opposite the sulcus limitans hippocampi lies a small group of cells which appear to be migrating into the marginal velum from the matrix. This group of cells is coincident with the extent of the limiting sulcus, and it is the primordial fascia dentata (figs. 29 to 31, *Fas. den.*). It lies wholly within the hippocampal formation above the limiting sulcus.

The remainder of the dorsal medial wall is histologically the same tissue as that composing the dorso-lateral one. It is the neopallium (figs. 29 to 32, *Neopal.*) and is characterized at this stage of development by a dense matrix and a marginal velum, filled with migrating neuroblasts. In amount it is proportionally and actually greater in the rostral than in the caudal division of the telencephalon.

The division of the ventro-medial sector into two areas, similar to those described in no. 940 (the 14-mm.), is clearly seen in the section immediately posterior to the level of the tuberculum olfactorium (fig. 31). The more dorsal slender wall is the septum ependymale (fig. 31, *Sept. epen.*), and the more ventral, the septum (fig. 31, *Sept.*). In the latter region a minute differentiation in the form of a row of cells lying in the marginal velum may be identified. This is the nucleus medialis septi, which also appears in levels through the tuberculum olfactorium (figs. 29 and 30, *Nuc. med. sept.*). This cell grouping is continuous

over the angulus terminalis with those cells already identified as the primordial fascia dentata, though these two cell masses originate in different sectors and have very different morphological significance. The lamina epithelialis, above the foramen interventriculare, has invaginated now to form the lateral choroid plexus.

The last sector, the ventro-lateral, shows plainly the beginnings of the medial and lateral limbs of the caudate complex. The separation of the caudate nucleus from the neopallium is made evident by a shallow ventricular groove. The angulus ventralis, due in the main to the enlargement of the ventricular eminence of the caudate's medial complex (figs. 30 and 31, *Cor. str. med.*), bounds the septum laterally.

The following histological changes have taken place between the stage last described, the 14-mm., and the 19.1-mm.:

1. The expansion of a mantle zone in the septal region.
2. The acceleration of growth in the neopallium, accompanied by the complete incorporation of the hippocampal anlage in the medial wall.
3. The appearance of the primordial fascia dentata opposite the sulcus limitans hippocampi. It is continuous with a similar group of cells in the septum, the nucleus medialis septi.
4. The appearance of the plexus chorioideus ventriculi lateralis.
5. The obvious indentation of the hippocampal wall, the fissura hippocampi.

The 20-mm. embryo, Mall Collection, 460 (figs. 17, 33 to 37)

The morphological and histological differentiation of the telencephalon of this embryo resembles in large part that of the 19.1-mm.; the boundary lines of the various areas remain unchanged and they occupy the same relative positions in the four sectors. The fissura hippocampi is a broad bow-shaped bend of the medial wall of the hemisphere involving practically the entire extent of the hippocampal formation and extending from the base of the bulbus olfactorius to the tip of the temporal pole (fig. 17, stippled area). Rostral to the terminal plate, the greatest depth of this fissure lies in the center of the hippocampal forma-

tion, but caudal to the lamina the greatest depth of this fissure shifts toward the sulcus limitans hippocampi (compare *Fis. hip.* in figs. 33 and 34 with the fissure in figs. 35 to 37). The depth of the fissure, then, is found in the center of the future hippocampus at those levels in which its ventral limb is continuous with the septum itself; when, however, its ventral limb is joined to the thin septum ependymale or the taenia fornicis, the depth of the fissure shifts ventrally.

Within the septum ependymale is now found a thin marginal velum (fig. 35). There is no change in the area intercalata. However, in the lamina epithelialis, the portion which extends to the paraphyseal arch (fig. 36) approaches the cuboidal epithelium of much older stages while that portion contiguous to the outer limb of the velum transversum is as primitive as the whole of the area in the 19.1-mm. embryo.

The primordium of the fascia dentata extends from a region anterior and dorsal to the angulus terminalis almost to the tip of the temporal pole, opposite the sulcus limitans hippocampi (cross-hatched area, fig. 17). In figure 33 (*Fas. den.*) it lies as a small well-defined group of cells in the marginal velum below the fissura hippocampi (fig. 33, *Fis. hip.*). Here, as well as in the next figure (fig. 34, *Fas. den.*), these cells seem to be continuous with the nucleus of the septum, the nucleus medialis septi (*Nuc. med. sept.*). This nucleus always appears ventral to the sulcus limitans hippocampi and can be identified easily in the next level pictured, through the septum ependymale (fig. 35, *Sept. epen.*). Here also the fascia dentata is well marked. In the last two levels presented this structure maintains its initial relationship to the sulcus limitans hippocampi and the fissura hippocampi (figs. 36 and 37).

The first three figures (figs. 33 to 35), when compared with those of the 14-mm., are excellent illustrations of the shifting of the hippocampal primordium to the midmedial region of the cerebral hemisphere. The whole dorsal lateral wall and almost half of the dorso-medial, at this stage, are composed of neopallium. But within the ventro-lateral sector bounded by the sulcus above the lateral limb of the caudate complex and the angulus

ventralis, that area which in mammals is said to represent the whole lateral half of the hemisphere evagination, only two cellular layers on the ventricular contour of the caudate nuclei and a diffuse cellular mass beneath them can be identified. Upon careful examination a thin layer of cells may be seen, swinging around the ventro-medial corner of the hemisphere in the marginal velum, the first evidence of the cortex of the tuberculum olfactorium. The relationship of tissue within the ventro-medial and ventro-lateral sectors is identical in the 19.1-mm. and 20-mm. embryos.

This embryo shows an advance over the last in:

1. The appearance of a clear zone in the septum ependymale.
2. Growth of neopallial tissue in the caudal and inferior aspect of the hemisphere.
3. The hippocampal anlage begins to assume its definite shape caudally, i.e., the caudal end of the hippocampal formation has advanced forward to define the temporal lobe (figs. 16 and 17).
4. The fascia dentata has differentiated as far as the temporal tip of hippocampal primordium.

The 27.8-mm. embryo, University of Chicago, H 91 (figs. 38 and 39)

The general relationships of the various components of the telencephalon are the same in this embryo as those described for the 20-mm. However, the intrinsic differentiation within the cortex has become evident. Within the neopallium two cell layers have migrated out of the matrix. The outermost layer is that of the pyramids (fig. 29, *Pyr. c. l.*), and one next the matrix is the intermediate cell layer (figs. 38, 39, *Poly. c. l.*). But in the hippocampus the latter group only has appeared. The cells occupy the dorsal lip of the hippocampal fissure. This differentiation is especially marked anterior to the paraphyseal arch. Here also, as before, the primordial fascia dentata (figs. 38, and 39, *Fas. den.*) lies opposite the sulcus limitans hippocampi (figs. 38 and 39, *Sul. vent.*).

The olfactory bulb is in the process of actual detachment, so that its separation from the ventral sectors in the rostral pole has produced the fissura prima in the medial wall. Differentiation characteristic of the neopallium appears in the dorsal lip of this fissure. There is in the other embryos, nos. H 173 (19 mm.) and 460 (20 mm.) a small area dorso-frontal to the region where the fila olfactoris enter, which does not show the differentiation characteristic of the hippocampal anlage.

The septum endymale is divided into two layers, an outer clear zone and an inner matrix (figs. 38 and 39, *Sept. epen.*). This same tectonic arrangement is characteristic also of that tissue which adjoins the midvault of the foramen interventriculare. The change from a simple epithelium to the two cell layers described seems to be the first step in the process of differentiation in the lamina terminalis, a process which proceeds caudally.

The progress of growth has added the following:

1. Differentiation of the neopallium into three cell layers.
2. Appearance of the intermediate cell layer in the dorsal part of the primordium hippocampi, a process whose development is more marked rostral to the terminal plate.
3. The septum endymale contains two cell layers throughout, thus differentiating it from the static area intercalata.

The 32.1-mm. embryo, University of Chicago, H 41
(figs. 40 and 41)

At the level of the root of the olfactory bulb (fig. 40) the cortical differentiation extends ventrally as far as the fissura prima. This groove with its dorsal differentiation continues for a short distance upon the rostral end of the medial wall. Caudal to the root of the olfactory bulb there is an area of unmistakably hippocampal formation. Here the intermediate cell layer has crept out of the matrix below the level of the fissura hippocampi and the pyramidal cell layer shows itself in the dorsal lip of the fissure. This migration is characteristic of the preterminal hippocampal formation. That part of the future hippocampus

which lies between the levels of the lamina terminalis and the velum transversum is an area transitional to the sickle-shaped postvelar tissue in which little or no cortical differentiation has taken place. It is in the latter that the fissure is best indicated. The fissure is best developed, then, in the least differentiated part of the hippocampus. The fascia dentata (fig. 41, *Fas. den.*) is now a continuous band of clumped cells opposite the sulcus limitans hippocampi. The fascia dentata is not coextensive with either this sulcus or with the hippocampal formation. However, the ventricular sulcus limitans hippocampi (*Sul. vent.*) seems to delimit its ventral extent except in that region where the anlage of the hippocampus gradually fades into the olfactory bulb.

The condition of the septum and septum ependymale is the same as that of H 91 (28.7 mm.), with the exception that its most dorsal portion is much wider measured antero-posteriorly.

The growth changes may be summed as follows:

1. More ventral differentiation of the preterminal portion of the hippocampal anlage with a coincident decrease in the depth of the fissura hippocampi, ventrally.
2. Further thickening of the outer layer in the septum ependymale.

The 39.1-mm. embryo, University of Chicago, H 163
(figs. 42 to 46)

At the level of the tuberculum olfactorium (fig. 42, cf. fig. 18, *tub. olf.*) the vault of the hemisphere from the corpus striatum laterally to the sulcus limitans hippocampi medially presents the typical cortical differentiation. Following the tissue which lies immediately dorsal to the sulcus limitans hippocampi to the level of the lamina terminalis, the typical cortical layers do not extend as far ventrally as the sulcus. In the marginal velum opposite it lies the fascia dentata (fig. 43, *Fas. den.*). This tissue is the most rostral limit of the hippocampus. The area immediately ventral to the hippocampus is that of the septum. Its growth is very marked in all directions. That growth is largely the result of differentiation of a new diffuse nucleus.

Already in the marginal velum in embryos H 173 and 460 a thin layer of cells, the nucleus medialis septi, was evident. Now, lying between this nucleus and the matrix is a large diffuse group of cells, the nucleus lateralis septi (fig. 43, *Nuc. lat. sept.*). Here, also, rostral to the anterior commissure, the separation of the ventro-lateral from the ventro-medial sector is emphasized by a prolongation of the angulus ventralis (fig. 43, *Ang. vent.*). Its accentuation in this embryo is due to the growth of the medial nucleus of the caudate complex. The ventricular angle marking the boundary between the dorso-lateral and the ventro-lateral sectors has become more acute because of changes in the corpus striatum, namely, a ventricular extension of the lateral root of the caudate complex and the appearance of the anlage of the lentiform nucleus (figs. 43 and 44, *Nuc. lent.*).

Examining the relationships of tissue at a level passing through the more caudal part of the lamina terminalis (fig. 44, *Lam. term.*) we find the septum is narrow. However, three groups of cells may be seen, the inner matrix, a middle diffuse group, the nucleus lateralis septi (fig. 44, *Nuc. lat. sept.*), and an outer thin layer, the nucleus medialis septi (fig. 44, *Nuc. med. sept.*). Their development will be considered in the second study. The hippocampus joins this tissue dorsally. Within the hippocampus the pyramidal cell layer extends past the middle of the fissura hippocampi, while the intermediate cell group lies in its ventral lip, just dorsal to the level of the sickle-shaped sulcus limitans hippocampi (fig. 43, *Sul. vent.*). Along the extreme ventral margin of the hippocampus extending dorsally from the region of the sulcus mentioned lies the fascia dentata (fig. 43, *Fas. den.*). In the space between the fascia dentata and the matrix lie the fornix fibers. These fibers also occupy the space between the medial and lateral septal nuclei. From this level, caudally, throughout the compass of the hippocampus, the fascia dentata grows dorsally along its most ventral margin (figs. 43 to 46, *Fas. den.*).

In figure 45, a section through the paraphysis, the lateral choroid plexuses join the lateral limbs of the paraphyseal arch (*Par. ar.*). The small tubules lying in cross-section on either

side of the arch are the rostral evagination of the roof of the third ventricle, the postvelar tubules of Warren (*P. vel. t.*). However, in the midline above the arch there are two small tubules whose continuity with the membranous roof is found anterior to the velum transversum. They form a real pouch which extends forward over the telencephalic roof plate (fig. 18, *Par. p.*). This condition of the human paraphysis is strikingly like that figured by Warren ('17) in the 25-mm. embryo (figs. 14 and 15). Rostral to the anterior limb of the paraphysis is the tela chorioidea telencephali medii. It is undifferentiated, a true epithelial tissue. The tissue, which joins it dorso-laterally, is separated into two layers, an inner matrix and an outer layer, which contains fibers, emerging from the hippocampus, the fornix (fig. 45, *For.*)

In a section parallel with the length of the fissura hippocampi (fig. 46, *Fis. hip.*) the fascia dentata (fig. 46, *Fas. den.*) is a thin band of cells lying in the marginal layer of the hippocampus. This section cuts the sickle-shaped fissura hippocampi tangentially so that the typical hippocampal structure may be seen at two ends.

The 43-mm. embryo, Mall Collection, 886 (figs. 47 to 50)

Orientation is difficult in this embryo, because it was cut coronally to the long axis of the cerebral evagination. A comparison of the levels from which the figures were taken with the median sagittal view (fig. 20) is illuminating. Figure 47 passes longitudinally through the rostro-dorsal extent of the fissura hippocampi. The fascia dentata lies as a band in the outer margin (*Fas. den.*). The intermediate cell layer is well differentiated, while that of the pyramids extends only across the margin of the hippocampus. Lying between the fascia dentata and the matrix are the developing fibers of the fornix system.

Figure 48 was taken at a level farther caudally, below the dorsal arch of the fissura hippocampi. The architecture of the hippocampus is the same in its anterior and posterior extent with the exception that the number of developing fornix fibers is greater posteriorly. Following the posterior limb to its caudal

end, there is no change in these relationships. Anteriorly, however, the cortical differentiation is coincident with the sulcus limitans hippocampi (fig. 49, *Sul. vent.*) and seems to be continuous with the same type of differentiation which lies dorsal to the root of the olfactory bulb. At both of these levels the septum contains three groups of cells as outlined in H 163 (39.1 mm.) and is separated from the hippocampus by the sulcus limitans hippocampi. There are no discernible parapyseal pouch or postvelar tubules.

Development in these two embryos may be summarized as follows:

1. Cortical differentiation in the region of the hippocampal formation, anterior to the parapyseal arch, and the coincident reduction in depth of the fissura hippocampi in this region.
2. The great growth of the lamina terminalis in all directions and an intrinsic differentiation into three cell groups, the septal nuclei.
3. The dorsal extension of the fascia dentata along the margin of the medial wall.
4. The development of the anterior commissure and the fornix system.

The eight brains described in the preceding pages have been arranged according to the time interval between the initiation of the growth process and its arrest, measured as the greatest length attained by the individual. The greater the difference in length the greater the growth changes. These embryos may be divided into the following groups:

1. The 11.8-mm.
2. The 14.0-mm.
3. The 19.1-mm. and the 20.0-mm.
4. The 27.8-mm. and the 32.1-mm.
5. The 39.1-mm. and the 43.0-mm.

This division is also a logical one, for when these groups are compared to the curve of change through which this particular protoplasm has passed, certain similarities are noticed, from which it seems that the recapitulation of phylogenetic processes is

woven into the fabric of their development in such a manner that later additions of brain substance have not erased the early history of the long passage. Consequently, there is a possible interpretation of early growth in the human telencephalon based upon a comparison of its growth with that attained by various representatives of the vertebrate phyla. However, it may appear when this study is complete that this so-called biological inertia which has been used to explain the striking similarities of development in the brains of the vertebrate series has its roots not in heredity as such, but in the more fundamental necessity of the mechanics of growth. Although certain recapitulations are complete, there is no stage of development whose rhythms of growth repeat in any way accurately earlier phylogenetic stages. If, however, the development of one particular tissue is watched with care, the sequence of intrinsic differentiation will appear in the order of a phylogenetic recapitulation. It is possible that the disturbing element is the growing neopallium, whose initial acceleration influences the growth rhythms of the other parts of the telencephalon and may therefore have modified the early phylogenetic relationships of this tissue.

DISCUSSION

Certain factors seem to be implicit in the growth changes of the telencephalon. They are the landmarks or points in the midline which show individual differentiation. These points are delimited by a peculiar histological structure and a characteristic external morphology. With such a series of stages as presented, the early development in the midline, together with those changes in the telencephalic vesicle whose various parts are confluent with them, may be followed.

Telencephalon medium

The midplane structures of the telencephalon medium have been divided into two main divisions by the angulus terminalis, the lamina terminalis and the area chorioidea. The lamina terminalis grows progressively thicker, rostro-caudally, and the thickening approaches the angulus terminalis progressively from

younger to older stages so far as studied. The area chorioidea is differentiated early into two regions, one of whose lateral limbs becomes the thin lamina epithelialis of the lateral plexus and the other an area in which changes are insignificant. The former is the paraphyseal arch and the latter is the tela chorioidea telencephali medii.

In table 4 the writer has attempted to measure, somewhat crudely to be sure, two dimensions of these midline structures. The total lengths of the telencephalon medium were taken by

TABLE 4
Measurements of divisions of the telencephalon medium

EMBRYO		TELENCEPHALON MEDIUM	LAMINA TERMINALIS		TELA CHORIOIDEA TELENCEPHALI MEDII		PARAPHYSIS	
Number	Length	Length	Length	Greatest width	Length	Width	Length	Width
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1121	11.8	2.388	0.94 ¹	0.054	0.248 ¹	0.02	1.24	0.02
940	14.0	1.620	1.048 ¹	0.088	0.168 ¹	0.028	0.404	0.032
H173	19.1	2.52	1.72	0.17	0.29	0.03	0.51	0.04
460	20.0	2.516	1.60	0.15	0.40	0.036	0.516	0.036
H 91	27.8	2.645	2.27	0.51	0.15	0.02	0.225	0.05
H 41	32.1	2.834	2.40	0.644	0.184	0.041	0.25	0.064
H163	39.1	3.24	2.56	0.80	0.21	0.72	0.470	0.044
886	43.0	3.34	2.94	0.73	0.15	0.75	0.2	0.045

¹ These are estimates, because the angulus terminalis is not present.

measuring the distance between the recessus preopticus and the velum transversum at the magnification of the model studied. That length was then divided by the magnification. Consequently, the figures are approximately the actual length in millimeters as found in the particular embryo and therefore comparative.

The distribution of the increase lies entirely in the lamina terminalis. The telencephalon medium grows in length mainly because the lamina terminalis increases in length. If the accompanying figures 1 to 6 be examined, especially figures 3 to 6 sketch 1 and 1d, through the ventral and dorsal divisions of the







terminal plate, the growth in depth and width is apparent. This change is a continuous one and, as far as the evidence presented in this paper is concerned, it seems to be one which accompanies the intrinsic growth and differentiation of the contiguous structures. Certain it is, that following the initial differentiation of the septum into matrix and marginal velum and the subsequent appearance of the two septal nuclei, the matrix layer becomes noticeably poor in cells. The whole seems to be a growth at the expense of the cells in situ and not a migration of cells from other centers. In the two oldest embryos only, the anterior

ABBREVIATIONS

<i>A.ch.</i> , area chorioidea	<i>Hip.</i> , hippocampus
<i>A.ep.</i> , area epithelialis	<i>Hypoth.</i> , hypothalamus
<i>A.int.</i> , area intercalata	<i>Inf.</i> , infundibulum
<i>Ant.com.</i> , anterior commissure	<i>Is.cal.</i> , islands of Calleja
<i>Ang.term.</i> , angulus terminalis	<i>Lam.ep.</i> , lamina epithelialis
<i>Ang.vent.</i> , angulus ventralis	<i>Lam.ter.</i> , <i>Lam.term.</i> , lamina terminalis
<i>Ant.l.hyp.</i> , anterior lobe of hypophysis	<i>Lat.olf.tr.</i> , lateral olfactory tract
<i>Bul.olf.</i> , bulbus olfactorius	<i>Lim.men.</i> , limitans meningeae
<i>Cer.</i> , cerebellum	<i>Man.l.</i> , marginal velum
<i>Cor.mam.</i> , <i>Corp.mam.</i> , corpus mamillare	<i>Mat.</i> , matrix
<i>Cor.str.</i> , <i>Corp.str.</i> , corpus striatum complex	<i>Mes.</i> , <i>Mesen.</i> , mesencephalon
<i>Cor.str.lat.</i> , <i>Corp.str.lat.</i> , corpus striatum laterale	<i>Mes.f.pl.</i> , mesencephalic floor plate
<i>Cor.str.med.</i> , <i>Corp.str.med.</i> , corpus striatum mediale	<i>Mes.r.pl.</i> , mesencephalic roof plate
<i>Def.fas.den.</i> , fascia dentata	<i>Met.</i> , <i>Meten.</i> , metencephalon
<i>Dien.</i> , diencephalon	<i>Myel.</i> , myelencephalon
<i>Dien.r.pl.</i> , diencephalic roof plate	<i>Neopal.</i> , neopallium
<i>Di-tel.gr.</i> , di-telencephalic groove	<i>Nuc.ac.</i> , nucleus accumbens of Kappers
<i>Ep.ev.</i> , epiphyseal evagination	<i>Nuc.lat.olf.tr.</i> , nucleus lateralis tracti olfactorii
<i>Epith.</i> , epithalamus	<i>Nuc.lat.sept.</i> , nucleus lateralis septi
<i>Fas.den.</i> , fascia dentata	<i>Nuc.lent.</i> , nucleus lentiformis
<i>F.b.op.ch.</i> , bed of the optic chiasma	<i>Nuc.med.sept.</i> , nucleus medialis septi
<i>Fil.olf.</i> , fila.olfactoria	<i>Olf.bulb.</i> , olfactory bulb
<i>Fis.hip.</i> , fissura hippocampi	<i>Olf.evag.</i> , evagination of olfactory bulb
<i>Fis.pr.</i> , fissura prima	<i>Op.c.</i> , optic chiasma
<i>For.</i> , fornix	<i>Op.evag.</i> , <i>Opt.evag.</i> , optic evagination
<i>For.int.</i> , foramen interventriculare	<i>Op.s.</i> , <i>Op.st.</i> , optic stalk
<i>Hab.</i> , habenula	<i>Par.ar.</i> , parapyseae, arch
<i>Hab.com.</i> , habenular commissure	<i>Par.p.</i> , parapyseal pouch
	<i>p.c.</i> , pars crassa of lamina terminalis
	<i>P.com.</i> , <i>Post.com.</i> , posterior commissure

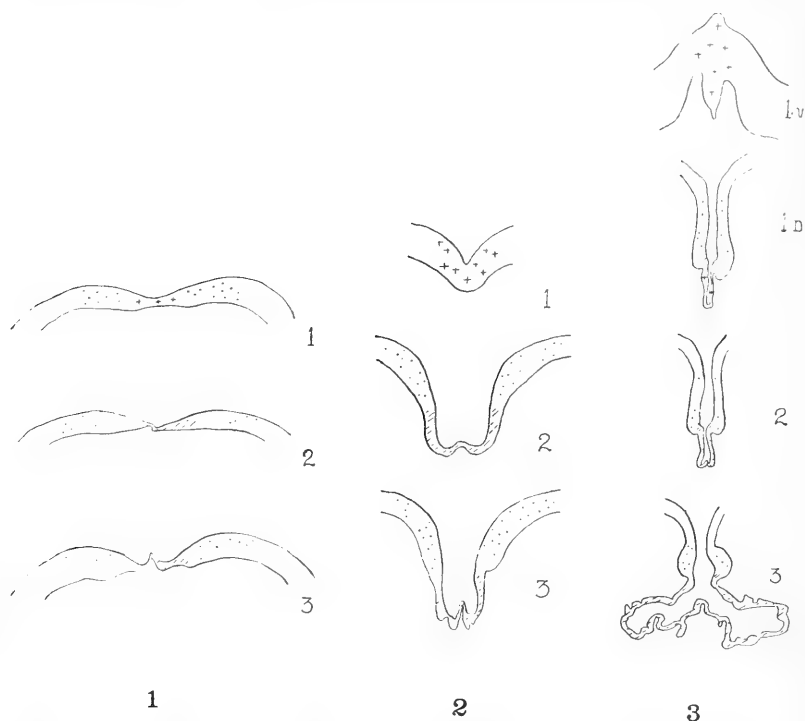
<i>Pl.ch.vent.lat.</i> , plexus chorioideus ventriculi lateralis	<i>Sept.epen.</i> , septum ependymale
<i>Pl.ch.vent.quart.</i> , <i>Pl.ch.v.quar.</i> , plexus chorioideus ventriculi quarti	<i>Sul.dors.</i> , sulcus dorsalis thalami
<i>Pl.ch.vent.ter.</i> , plexus chorioideus ventriculi tertii	<i>Sul.fim.den.</i> , sulcus fimbrio-dentatus
<i>Poly.c.l.</i> , intermediate layer of migrating neuroblasts	<i>Sul.fu.pl.ch.vent.lat.</i> , sulcus futurus plexus chorioidei ventriculi lateralis
<i>Post.hyp.</i> , <i>Post.l.hyp.</i> , posterior lobe of hypophysis	<i>Sul.lim.</i> , sulcus limitans
<i>P.Ra.</i> , <i>P.Rat.</i> , pouch of Rathke	<i>Sul.mon.</i> , sulcus Monroi
<i>Prim.hip.</i> , primordium hippocampi	<i>Sul.vent.</i> , sulcus limitans hippocampi
<i>p.t.</i> , pars tenuis of lamina terminalis	<i>Tel.</i> , telencephalon
<i>P.vel.r.</i> , postvelar arches	<i>Tel.ch.tel.med.</i> , tela chorioidea telencephali medii
<i>P.vcl.t.</i> , postvelar tubules	<i>Tel.evag.</i> , telencephalic evagination
<i>Pyr.c.l.</i> , pyramidal cell layer	<i>Tel.r.pl.</i> , telencephalic roof plate
<i>Rec.inf.</i> , recessus infundibuli	<i>Tc.pl.ch.vent.lat.</i> , taenia plexus chorioidei ventriculi lateralis
<i>Rec.mam.</i> , recessus mamillaris	<i>Te.pl.ch.vent.quar.</i> , taenia plexus chorioidei ventriculi quarti
<i>Rec.op.</i> , optic ventricle	<i>Te.pl.ch.vent.ter.</i> , taenia plexus chorioidei ventriculi tertii
<i>Rec.postop.</i> , recessus postopticus	<i>Thal.</i> , thalamus
<i>Rec.preop.</i> , recessus preopticus	<i>Tub.cin.</i> , tuber cinereum
<i>Reg.par.ar.</i> , region of the parapyseal arch	<i>Tub.olf.</i> , tuberculum olfactorium
<i>Reg.sept.</i> , region of the septum ependymale	<i>Undif.f.den.</i> , <i>Undif.fas.den.</i> , undifferentiated fascia dentata
<i>Rhom.fos.</i> , rhomboid fossa	<i>Vel.trans.</i> , velum transversum
<i>Sept.</i> , septum	<i>Ven.lat.</i> , <i>Vent.lat.</i> , ventriculus lateralis
	<i>Vent.ter.</i> , ventriculus tertius

EXPLANATION OF SYMBOLS

	hippocampus.
	fascia dentata
	area epithelialis
	subcortical olfactory centers
	septal nuclei
	sulcus limitans hippocampi

commissure has grown across the midline. The bed for these fibers is laid down long before the fibers themselves appear. Further, the growth is appositional, taking place ventral to the center of the main body of the terminal plate and progressing both dorsally and ventrally. The most marked growth is found in the former region, and not in the vicinity of the *angulus terminalis* or the *recessus preopticus*.

There is no constant change in the non-plexiform portion of the *area chorioidea* and if the structures adjoining this region are examined (figs. 1 to 6, sketch 2) they appear to be constant.



Figs. 1 to 6 These are pen sketches of the midregion of transverse sections of the telencephalon, showing the position of cell groups in the tissue; they were drawn either from photographs of the sections or from projections made with the Edinger apparatus.

Fig. 1 The 11.8-mm. embryo, no. 1121, Mall Collection. $\times 25$

Fig. 2 The 14.0-mm. embryo, no. 940, Mall Collection. $\times 25$

Fig. 3 The 19.1-mm. embryo, H 173, University of Chicago. $\times 16\frac{2}{3}$.

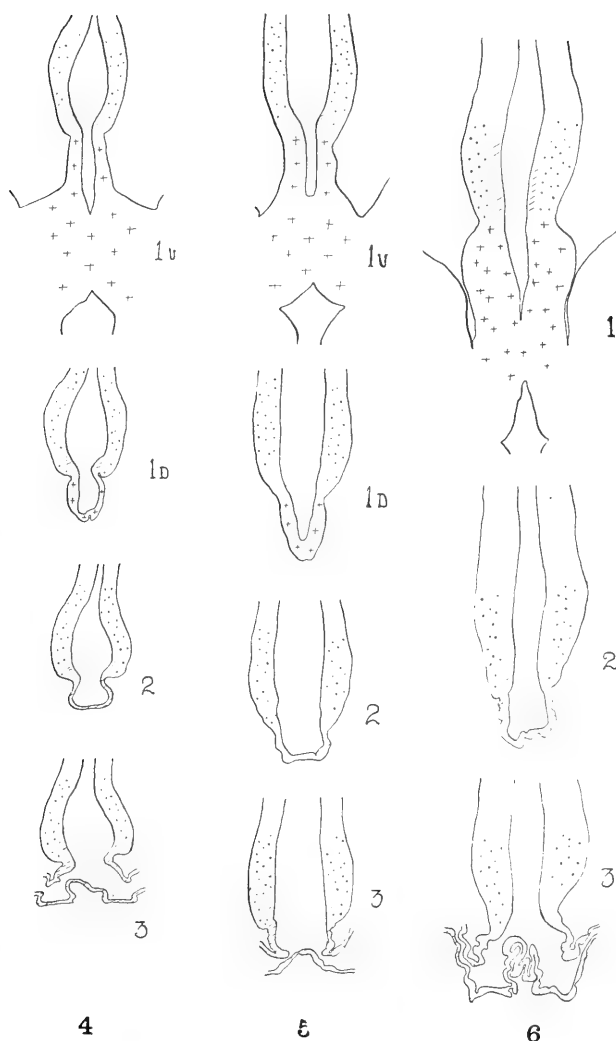


Fig. 4 The 27.8-mm. embryo, H 91, University of Chicago. $\times 12\frac{1}{2}$.

Fig. 5 The 32.1-mm. embryo, H 41, University of Chicago. $\times 12\frac{1}{2}$.

Fig. 6 The 39.1-mm. embryo, H 165, University of Chicago. $\times 12\frac{1}{2}$.

The levels from these various embryos are designated as follows: 1) through the lamina terminalis; 1 v) through the ventral part of the lamina terminalis; 1 d) through the dorsal part of the lamina terminalis; 2) through the tela chorioidea telencephali medii; 3) through the paraphysis.

The various regions of the medial wall contiguous to the midline structures are self-explanatory, see the legend below the list of abbreviations.

The only deduction which the present series allows is that the growth in this region is immaterial. In such a case this measurement lends another landmark to the study of the midline. Probably the most striking group of figures presented are those of the parapyseal arch. There is little change in the antero-posterior length; in this particular series, the younger embryos possess the longer arches. The shortening of the distance between the



Fig. 7 Medial sagittal view of a model of a 11.8-mm. embryo belonging to the Mall Collection in Baltimore, no. 1121. $\times 16\frac{2}{3}$.

anterior and the posterior limb coincides with the decrease in width and the formation of the actual plexus itself. The constant feature is not the pouch; it is rather the simple arch itself. If these figures are substantiated by subsequent work, the parapyseal arch will be a more prominent feature of the younger stages than of the older ones. This finding seems to place the human embryo in phylogenetic line with other mammals, the difference being not in its relative extent, but in the complexity of its bizarre outpouchings. This does not disagree with the work

of Warren and Bailey; it simply calls attention to a fact not recognized before, that the paraphyseal arch is present even in very young embryos in a simple form, extensive when compared to the whole midline, especially in these brains. It of necessity retains its fundamental importance as a midline structure.

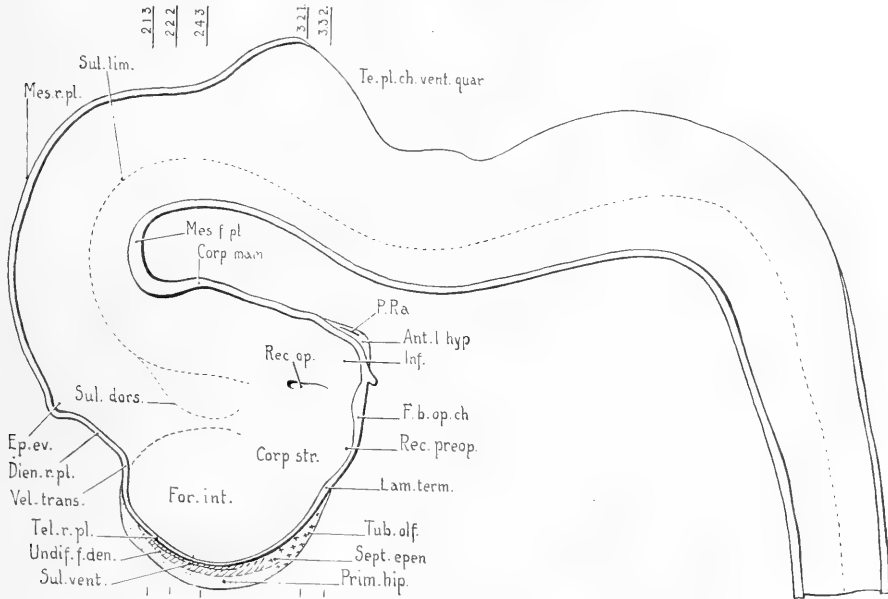


Fig. 8 This is a pen-and-ink outline of the same model as that shown in figure 7. Histologically distinct areas in the telencephalic medial wall are projected in their relative positions upon its surface. The key to these areas as well as those of the other models will be found below the list of abbreviations (p. 121). The annotations on all of the models refer to numbers of specific sections, whose photographs are reproduced in this paper or will appear in those which follow.

If these midline structures prove to be the landmarks which the writer has shown them to be in the embryos so far studied (and certainly all the well-preserved embryos between the ages mentioned which have been studied in the Department of Anatomy at Chicago and the Mall Collection in Baltimore substantiate that decision), the growth of the telencephalon adjoining them may be followed accurately. Herein lies an approach to data which

will enable the growth of the medial hemisphere wall to be interpreted more wisely. It may also give new data upon the development of the new parts of the cortex lying on the medial wall of the hemisphere.

Area epithelialis

The area epithelialis lies between the telencephalon medium and the sulcus limitans hippocampi. In the 11.8-mm. embryo this tissue is almost negligible in the rostral division of the hemisphere, but it becomes markedly greater in the caudal portions of that evagination. This difference is one of fundamental importance (figs. 1 to 6, *sketches 2 and 3*). Along the dorsal margin of the area epithelialis on the opposite side of the sulcus limitans hippocampi, the fornix is developed. This sulcus is regarded as the ventral limit of the cerebral cortex and the epithelial tissue is the derivative of the most dorso-medial portion of the roof of the primitive evaginated telencephalon. In such a primitive condition, the fornix formed a fringe along its lateral border and the anlage of the hippocampus lay laterally of it (i.e., morphologically ventral to it). This condition is almost exactly realized in the brain of *Ichthyomyzon concolor* (Herrick and Obenchain, '13). In the process of evagination the relations of the area epithelialis to the fornix and the hippocampus are reversed; that is, the two latter areas are turned first outward, then upward, so that in the medial wall of the hemisphere they come to lie dorsally of the area epithelialis. This seems to be a true statement of the case, because in the 11.8-mm. brain the sulcus limitans hippocampi is coextensive with the epithelial

Fig. 9 This is the anterior view of the model shown in figures 7 and 8, no. 1121 of the Mall Collection. $\times 16\frac{2}{3}$. There is a slight depression in the midline separating the initial evagination into two halves.

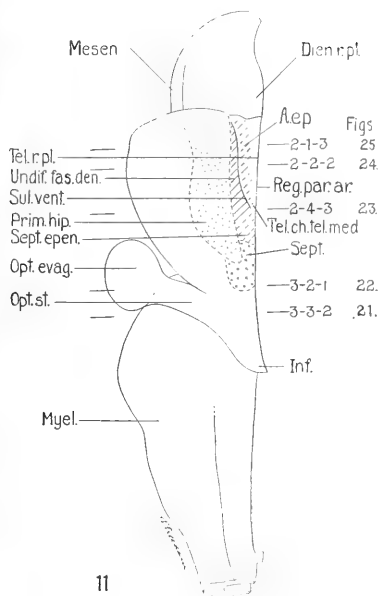
Fig. 10 This is the anterior view of the same model as that shown in figures 13 and 14. This 14-mm. embryo belongs to the Mall Collection in Baltimore, no. 940. $\times 16\frac{2}{3}$.

Fig. 11 This is a pen-and-ink sketch of the same view as that shown in figure 9, no. 1121. $\times 16\frac{2}{3}$. The areas indicated in figure 8 are shown in their dorsal extent. The planes of section of figures 21 to 25 are indicated.

Fig. 12 Anterior view of no. 940, same embryo as figure 10. $\times 16\frac{2}{3}$.



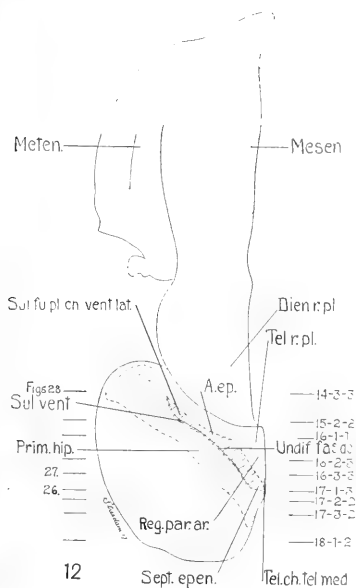
9



11



10



12

tissue and approaches the extent of the differentiation in the hemisphere wall termed the primordial hippocampus (figs. 8 and 24). The interpretation of the further development of this tissue must be correlated with this finding, as well as with the facts of its phylogenetic development.

This region of generalized morphology and histology was divided for descriptive purposes into three areas, entirely upon the

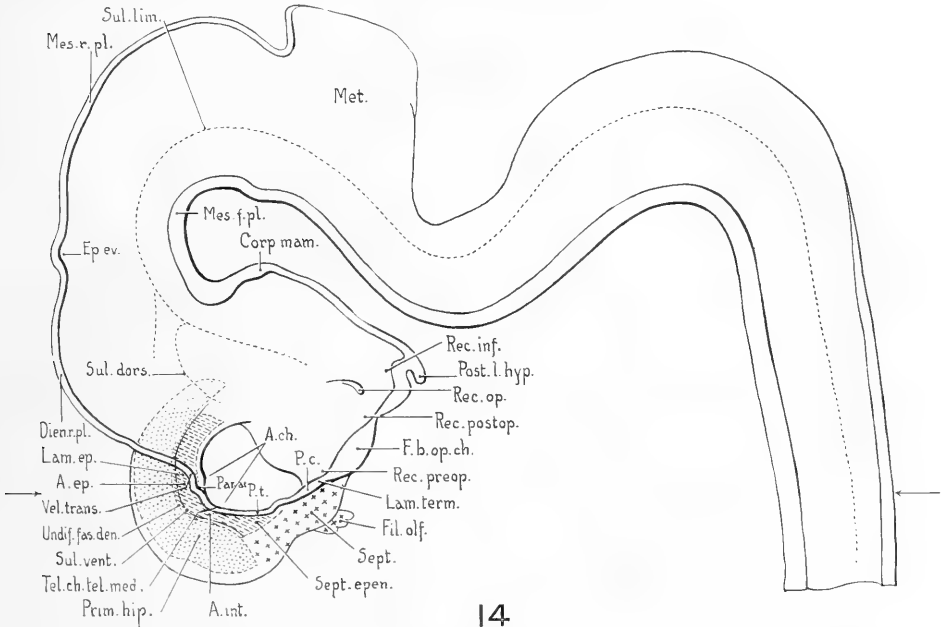


13

Fig. 13 Medial sagittal view of the model of brain no. 940, 14 mm. $\times 16\frac{2}{3}$.

basis of their respective destinies, namely, the septum ependymale, the area intercalata, and the lamina epithelialis. The relative growth of the septum ependymale may be seen at a glance by comparing the sections marked *1d* in figures 3 to 5. This region, an undifferentiated epithelium in the brains of the first four embryos described, begins to show a marginal velum in the 20-mm. embryo, which persists and grows in width in the brains of this group. The differentiation proceeds towards the angulus

terminalis as a limit. There is always some tissue, therefore, which retains the initial two layers of the early conditions. The area intercalata changes little in morphology. Its extent increases most noticeably in the dorso-ventral direction. Intrinsic differentiation is practically identical throughout the older brains of the series (19.1 mm. to 43 mm.).



14

Fig. 14 Pen-and-ink outline of the same brain taken from the same point of view as that shown in figure 13. The extent of the medial olfactory areas is projected upon the medial wall of the telencephalon. The arrows indicate the plane of section.

The lamina epithelialis lying between the lateral limb of the parapyseal arch and the sulcus limitans hippocampi increases in relative importance in the older embryos. In the 11.8-mm. the amount of epithelial tissue adjoining the lateral limb of the parapyseal arch is more extensive than that adjoining the unarched portion of the roof of the telencephalon medium. There is no lateral choroid plexus here; neither is the telencephalon more than a slight evagination. However, in the 14-mm. embryo

the paraphyseal arch is a definite dorsal protrusion of the roof plate just anterior to the velum transversum. The tissue contiguous to it is convex lateralward, the first indication of the invagination of the lateral choroid fissure. In all the rest of the series, the plexus exists as described for man by Bailey ('16a), namely, the choroid plexus of the lateral ventricle consists of two divisions, an anterior, whose lateral taenia is that of the fornix and whose medial is the lateral limb of the paraphyseal

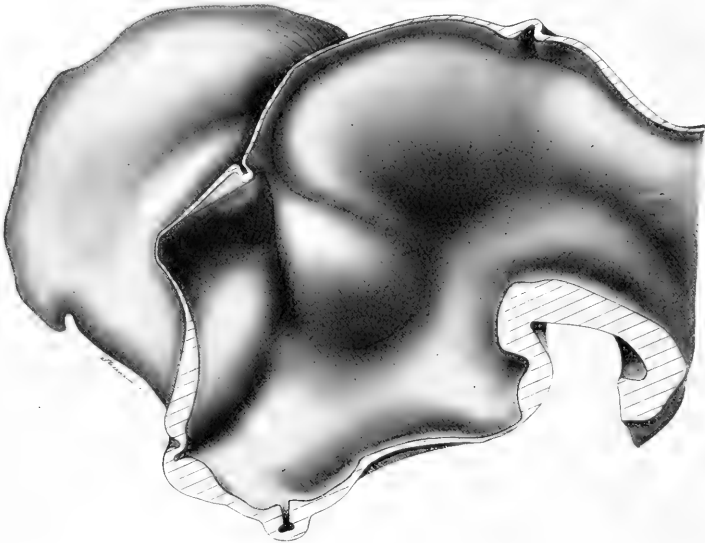


Fig. 15 Median sagittal view of the forebrain of H 173, a 19.1-mm. human embryo, belonging to the Chicago collection. $\times 16\frac{2}{3}$.

arch, and a posterior, whose lateral taenia is the same as that of the anterior division, but whose medial taenia is the telencephalic limb of the di-telencephalic groove, the taenia chorioidea. In these older embryos the hippocampus forms a complete crescent dorsal and lateral to the plexus. In all stages the primordial hippocampus precedes the choroid plexus as the hemisphere grows caudally.

Bailey ('16a, fig. 15, *a. c. p.*) has suggested that the growth of the posterior limb of the lateral choroid plexus is a simple

continuous invagination of tissue which lay undifferentiated in the sickle-shaped telencephalic wall adjoining the di-telencephalic groove. There is no indication in the 11.8-mm. or in the 14-mm. embryo of an area peculiarly differentiated in that region. The primitive hippocampus arches over the caudal pole of the developing hemisphere. Since the hippocampal tissue precedes

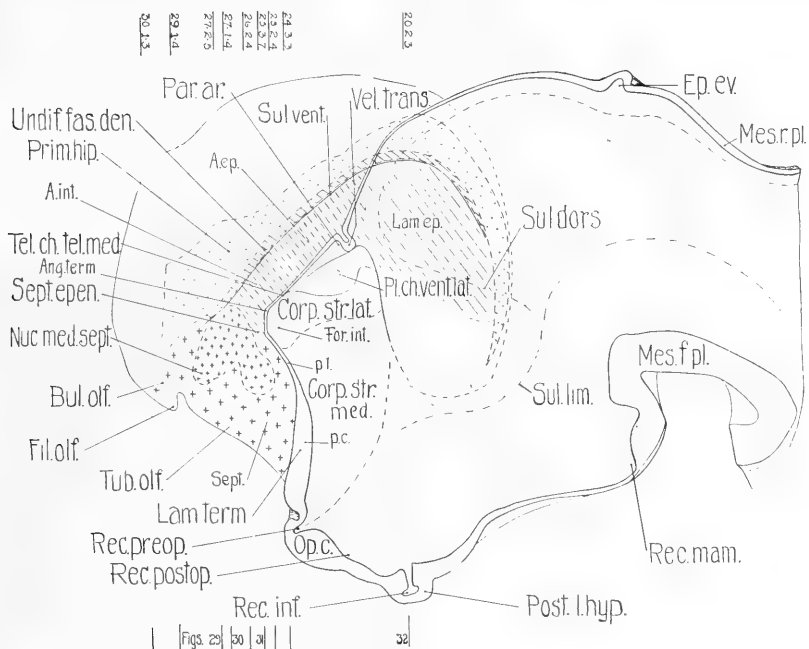


Fig. 16 Outline sketch of the same brain as that shown in figure 15. The dotted line, continuous with the telencephalon above the diencephalic roof plate, indicates the most caudal boundary of the cerebral hemisphere. The planes of section of figures 29 to 32 are indicated.

the choroid plexus in differentiation, it is natural to place the anlage of the plexus in the epithelial tissue which lies between the definitive hippocampus and the parapyseal arch and velum transversum. These two divisions of the lateral choroid plexus are fundamentally the same and their development coincides with the ventro-caudal growth of the hippocampus. The more extensive that growth is, the more extensive the so-called pos-

terior limb, and the less extensive, the less that portion will be developed. Bailey ('16b) says, discussing the morphogenesis of the choroid plexus:

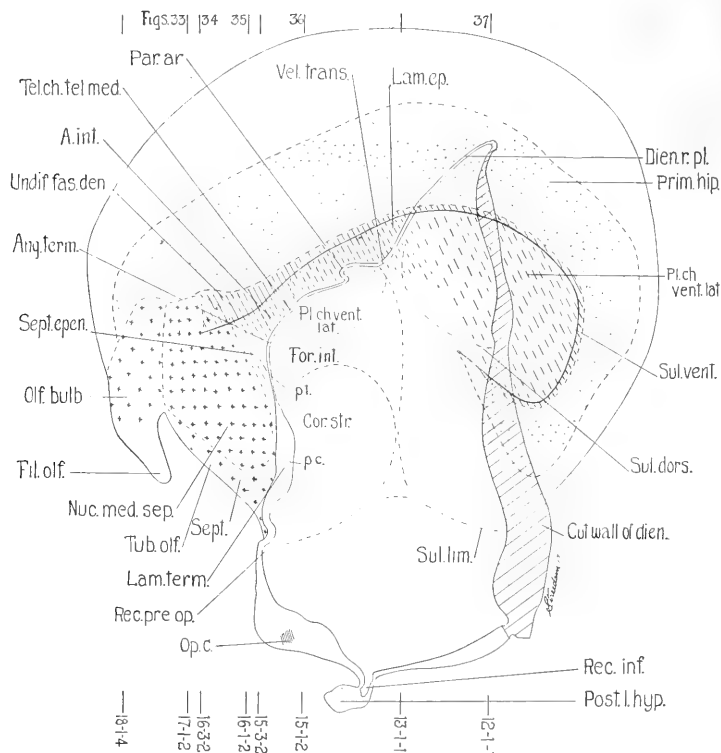


Fig. 17 This is an outline sketch of the brain of a 20-mm. embryo, no. 460, belonging to the Mall Collection. The area indicated by stippling corresponds to the extent of the fissura hippocampi. The planes of section of figures 33 to 37 are indicated.

In all forms below *Chelonia*, the lateral telencephalic plexus develops in what I have called elsewhere (Bailey, '16 a) the anterior lateral telencephalic chorioideal area, in the roof plate of the telencephalon between the paraphysis and the taenia fornicis of the medial hemisphere wall. With *Chelonia* comes a change. The lateral plexus arises in the anterior area chorioidea lateralis telencephali, as has been previously described, but in its later development crosses the taenia fornicis and invaginates also the posterior area chorioidea lateralis telencephali in the medial hemisphere wall. . . . This involvement of the medial hemisphere comes more and more to predominate in the develop-

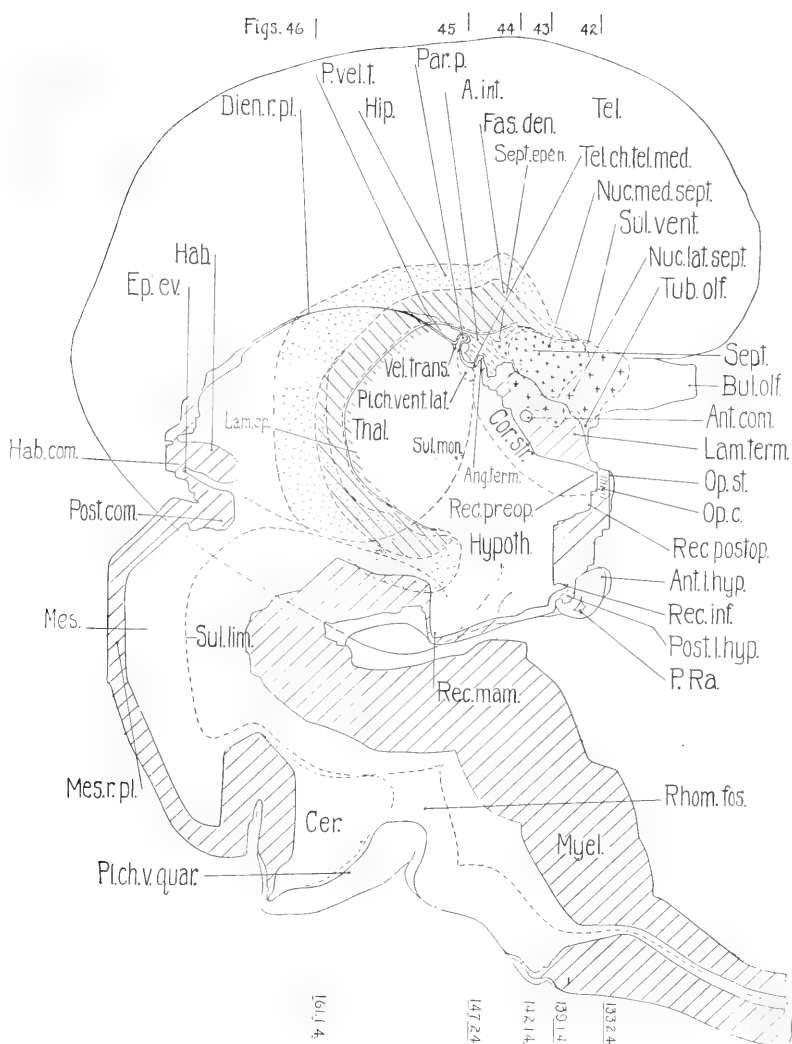


Fig. 18 Median sagittal view of the wax model made from the brain of a 39.1-mm. human embryo H 163 of the Chicago collection. $\times 8\frac{1}{2}$. The positions of the sulcus limitans and the thalamic midgroove are indicated by broken lines. The olfactory area is identified on the medial wall and sketched as if the thalamic wall were transparent. The planes of section of figures 42 to 46 are indicated.

ment of the lateral plexus as the hemispheres come more and more to dominate the development of the telencephalon (pp. 526 and 527).

However, it dominates only because of the progressively increasing importance of the portion of the hippocampus which is developing in the ventro-caudal direction concomitant with the growth of the hemisphere in that same direction to form the

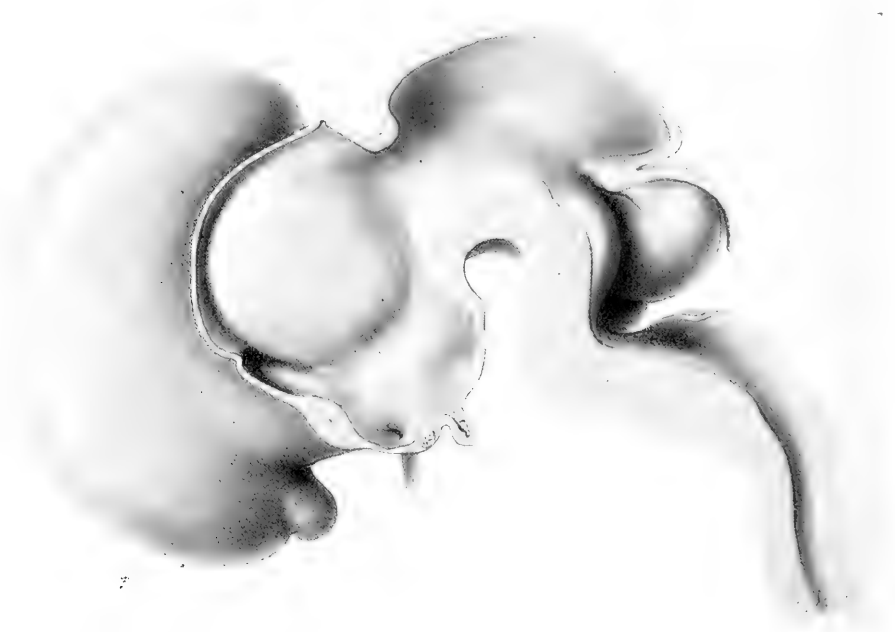


Fig. 19 Median sagittal view of a model made from the brain of a 43-mm. human embryo belonging to the Mall collection, no. 886. $\times 6\frac{2}{3}$.

temporal lobe. It seems more reasonable, therefore, to place the anlage of the telencephalic lateral plexuses, even in man, in the epithelial tissue which lies between the taenia fornicis or the sulcus limitans hippocampi and the paraphyseal arch and velum transversum and limited rostro-caudally by the anterior limb of the paraphyseal arch and the velum transversum. From this dorsally placed anlage it grows ventro-caudally. If this be true, the primordium of both divisions of the two lateral choroid plexuses lies immediately contiguous to the roof plate of the early unevaginated telencephalon.

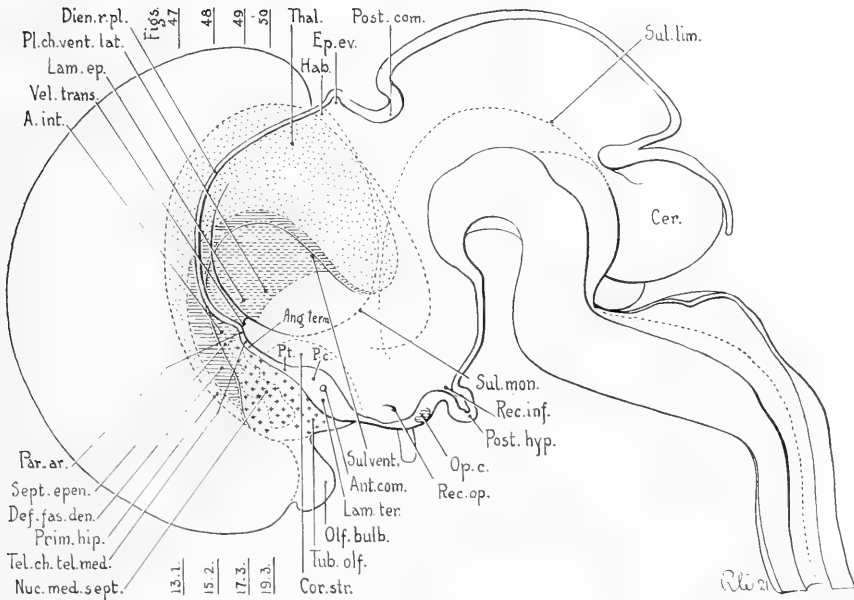


Fig. 20 Outline sketch of the same model as that shown in figure 19. The broken lines indicate the position of the sulcus limitans, the most caudal boundary of the telencephalic evagination, and the separation of the thalamus from the hypothalamus. The extent and the boundaries of the olfactory area projected upon the medial wall of the telencephalon can be seen through the main body of the thalamus. The planes of figures 47 to 50 are indicated.

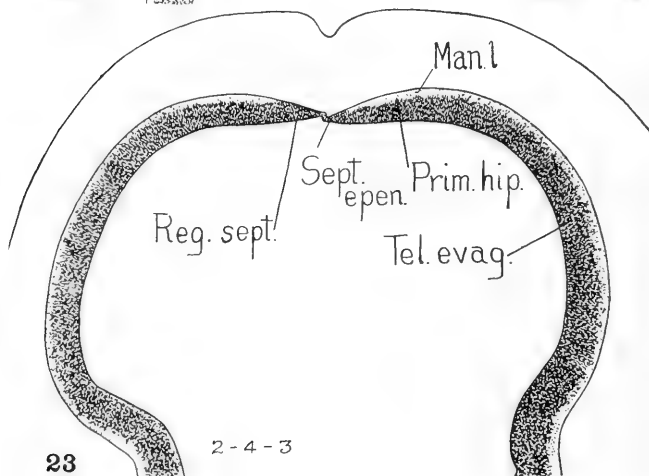
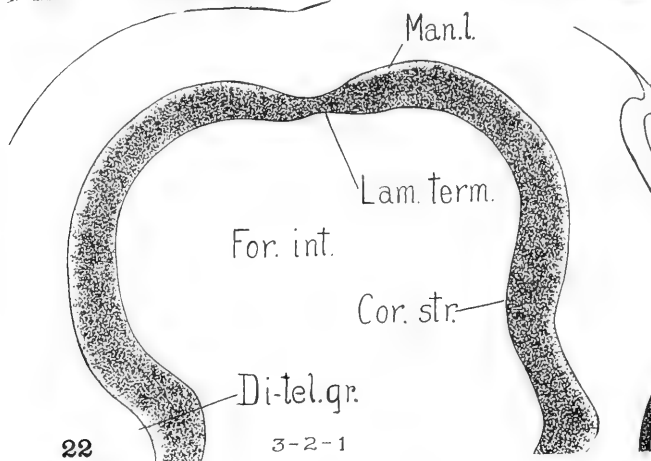
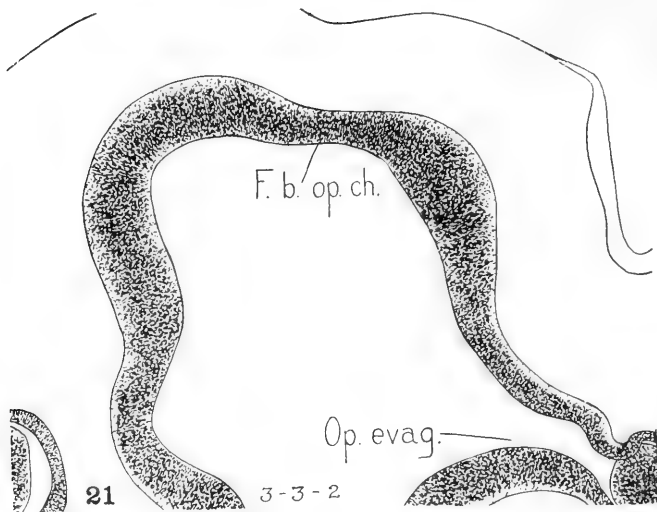
Figs. 21 to 25 A series of transverse sections, pen-and-ink outlines, made with the Edinger apparatus, through the various levels of the telencephalon of no. 1121, the 11.8-mm. embryo. The numbers below the sections correspond to those in figures 8 and 11 and each indicates a specific section. $\times 50$.

Fig. 21 At the level of the optic evagination.

Fig. 22 Through the middle of the lamina terminalis.

Fig. 23 Through the tela chorioidea telencephali medii.

Fig. 24 Through the paraphysis. In this and the preceding figure the wall of each cerebral hemisphere shows four distinct zones. Beginning in the mid-plane they may be designated as follows: 1) A thin septal area, telencephalic roof plate; 2) A thicker homogeneous area, the septum ependymale, bounded laterally by a shallow ventricular sulcus, the sulcus limitans hippocampi; 3) A small area showing a narrow mantle layer emerging from the matrix, the future hippocampus; 4) An adjoining and still more lateral area where no clear mantle zone is visible, the neopallium.



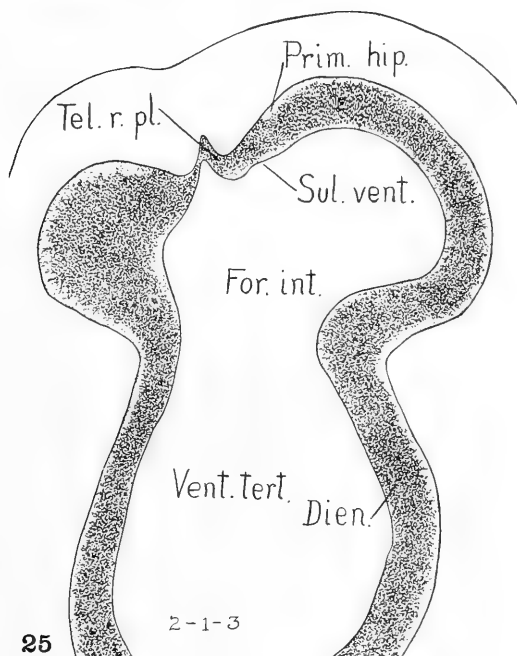
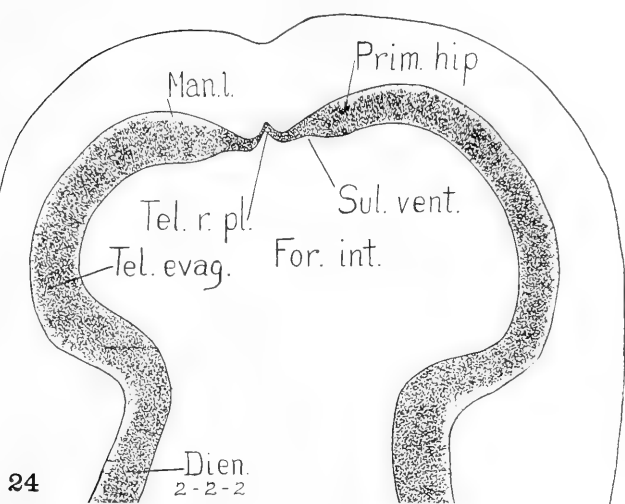
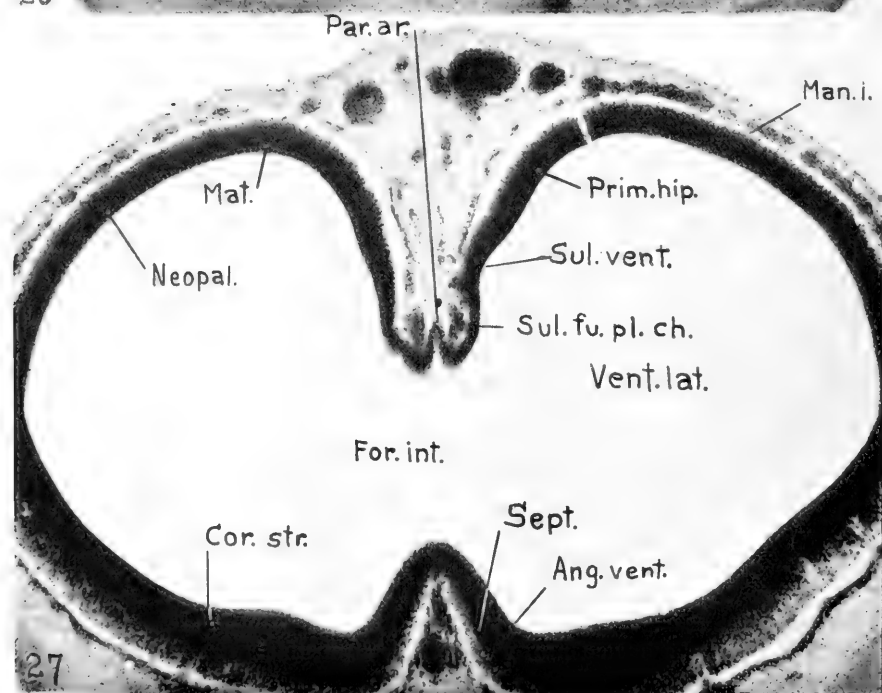
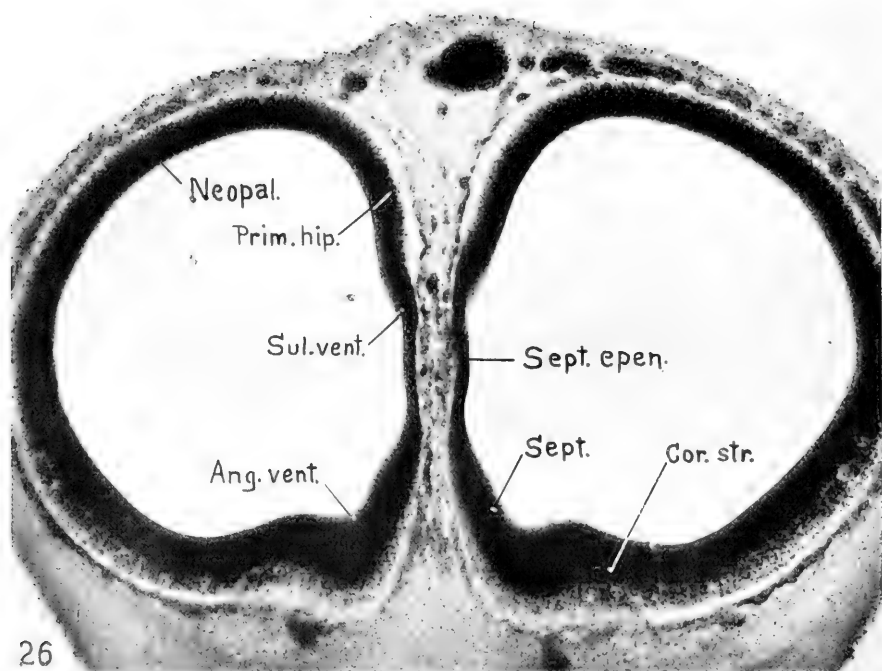
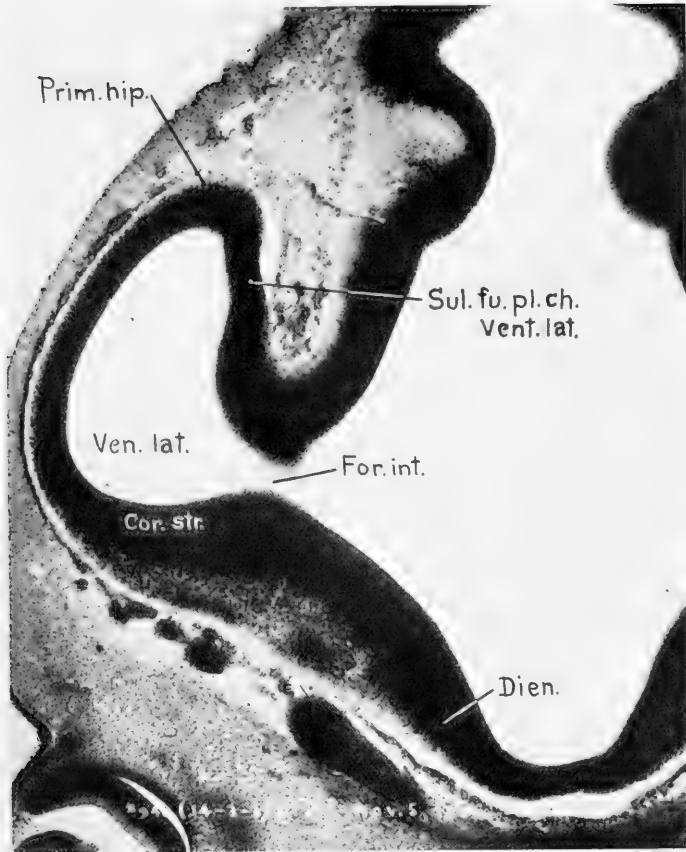


Fig. 25 This is taken slightly cephalad on the left, but passes through the paraphysis on the right. Upon the right the differentiation of the dorsal telencephalic wall may be divided into four histologically distinct areas as seen in figures 23 and 24.





Figs. 26 to 28 Photographs of three transverse sections through the 14-mm. embryo, no. 940. Two views of a model of this brain are shown in figures 10, 12, 13, and 14 and planes of sections are illustrated in figure 12.

Fig. 26 Section just anterior to the lamina terminalis and the tela chorioidea telencephali medii. The division of the medial wall into three regions is evident, namely, 1) a dorsal or neopallial; 2) a dorso-medial or hippocampal; 3) a ventral or septal.

Fig. 27 Through the paraphysis. In comparing this with figure 24 of no. 1121, the same upward curve in the membranous roof and the division of the dorsal half of the hemisphere vesicle into four regions may be noted. $\times 60$.

Fig. 28 Through the di-telencephalic groove. Note the thin marginal velum bordering the limitans meningeal on the dorsal aspect of the evagination in the primordial hippocampus. The massive undifferentiated area joining the diencephalon medially is the telencephalic limb of the di-telencephalic groove caudal to the choroid plexus evagination.

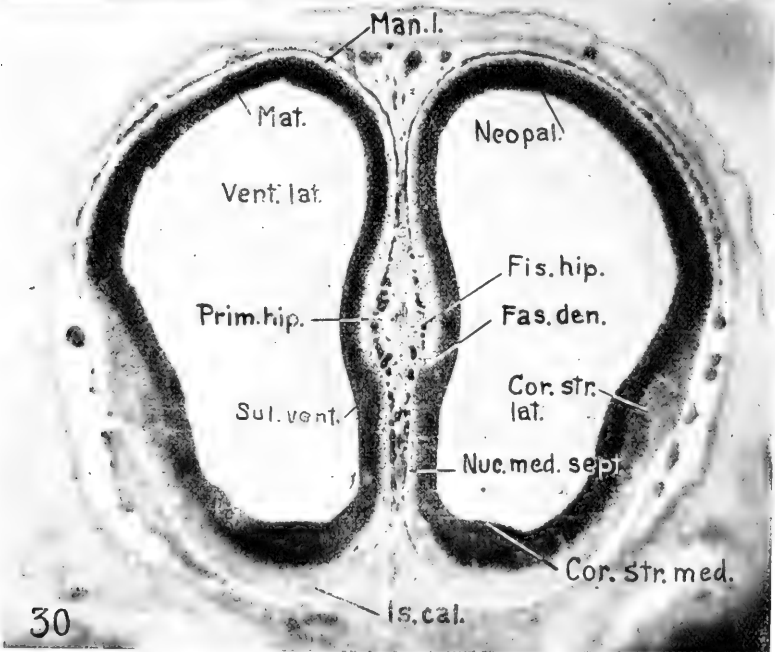
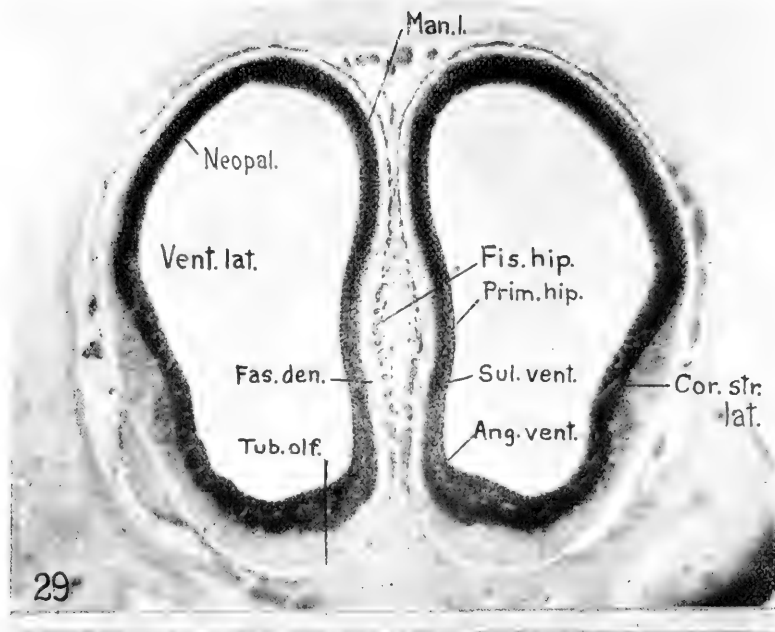


Fig. 29 Through the anterior part of the tuberculum olfactorium, showing the division of the medial wall into four histologically distinct areas, tuberculum olfactorium, septum, primordial hippocampus, and neopallium.

Fig. 30 Through the more caudal part of the tuberculum olfactorium. The region of the hippocampus is more accentuated, the groove is less shallow, the sulcus limitans hippocampi a more prominent feature. A group of cells, the fascia dentata, can be distinguished just above the sulcus.

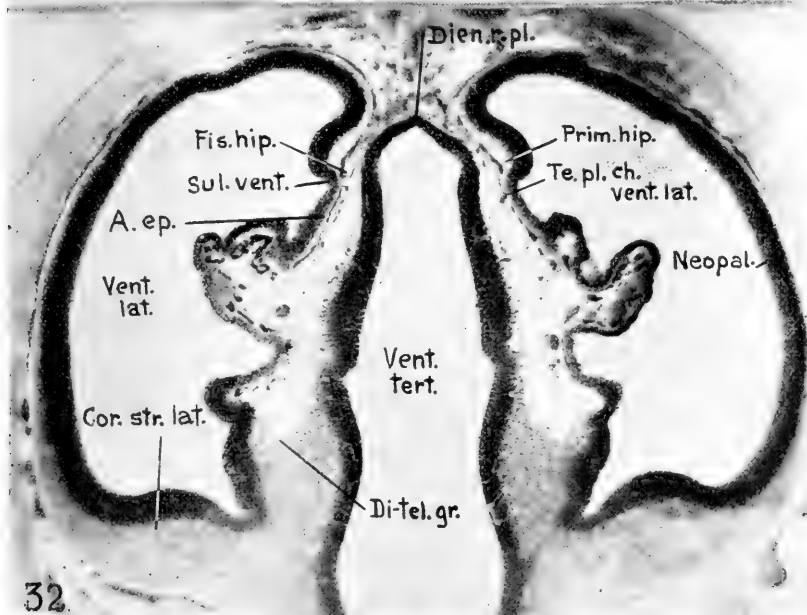
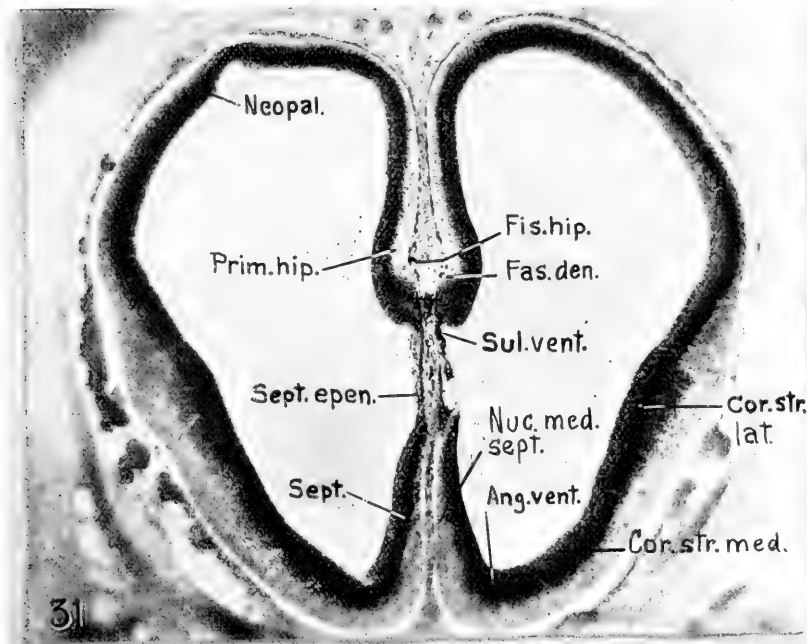
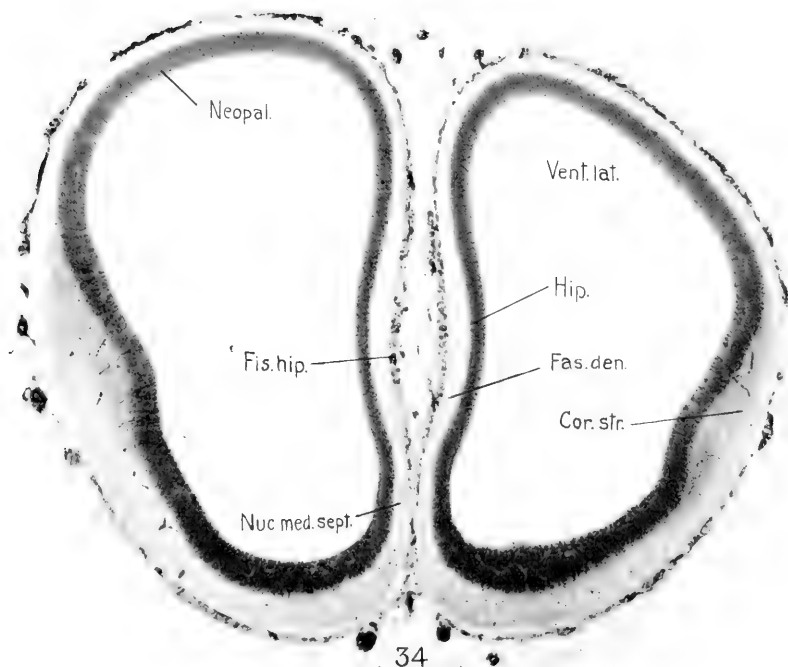
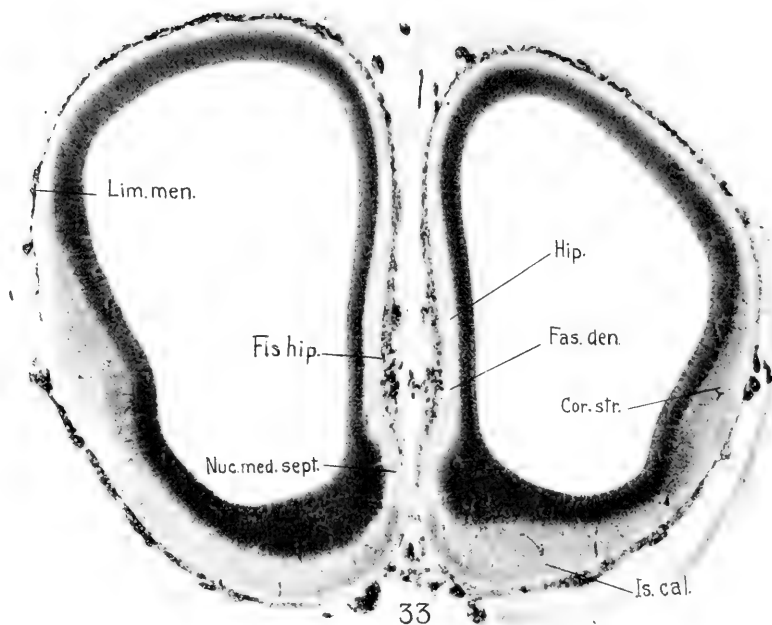


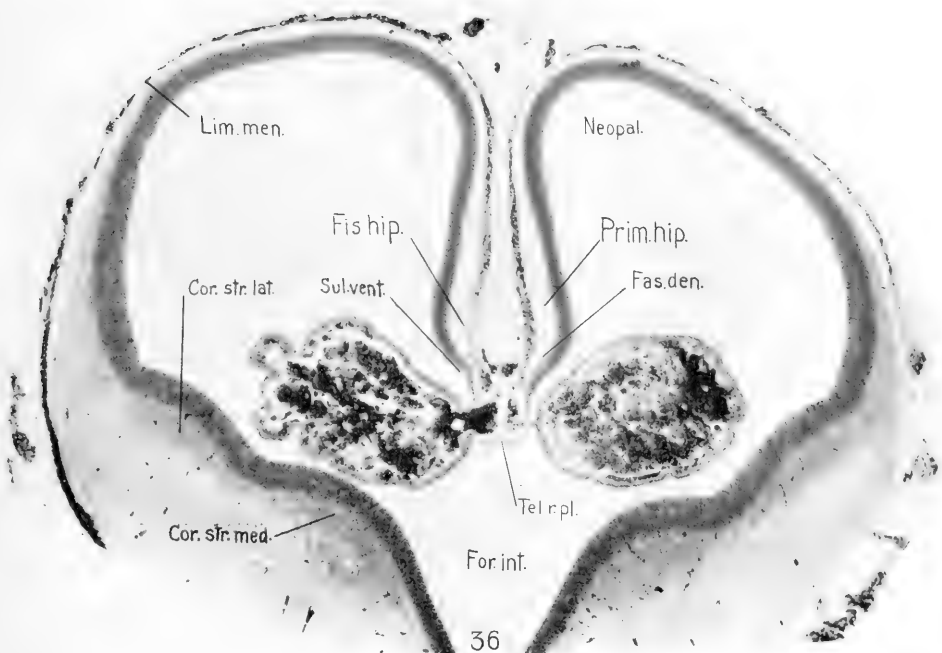
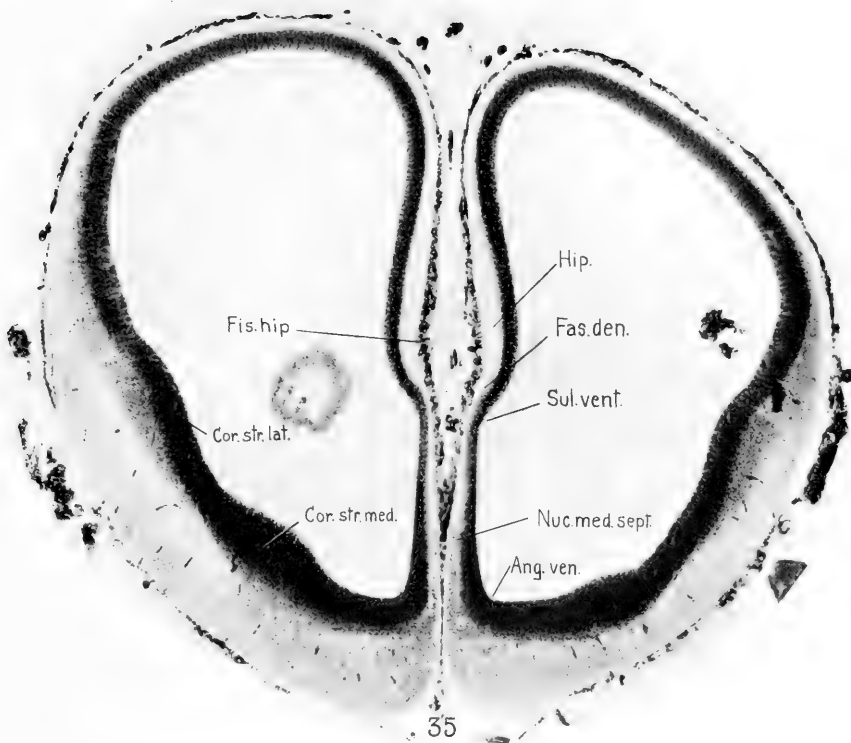
Fig. 31 Through the septum endymale, just rostral to the lamina terminalis region.

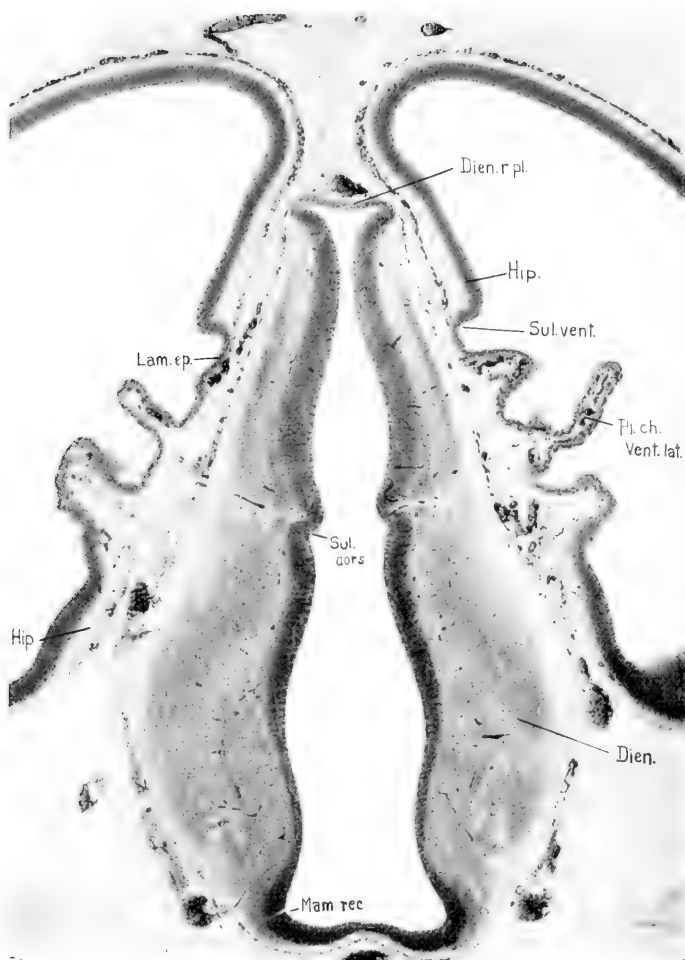
Fig. 32 Through the di-telencephalic groove, showing the invagination of the plexus chorioideus ventriculi lateralis, with the taenia fornicis.

Figs. 29 to 32 This series of reproductions through the forebrain was taken from embryo H 173, 19.1 mm., of the Chicago collection. This brain was cut transversely to the telencephalon. For the exact positions of these sections refer to figure 16. $\times 20$.



Figs. 33 to 37 These are photographs of selected sections from a transverse series of the telencephalon of a 20-mm. human embryo, no. 460, at the Carnegie Institution, the Mall Collection. $\times 25$. A model of the forebrain may be studied by turning to figure 17. The levels at which typical sections were photographed are indicated.





37

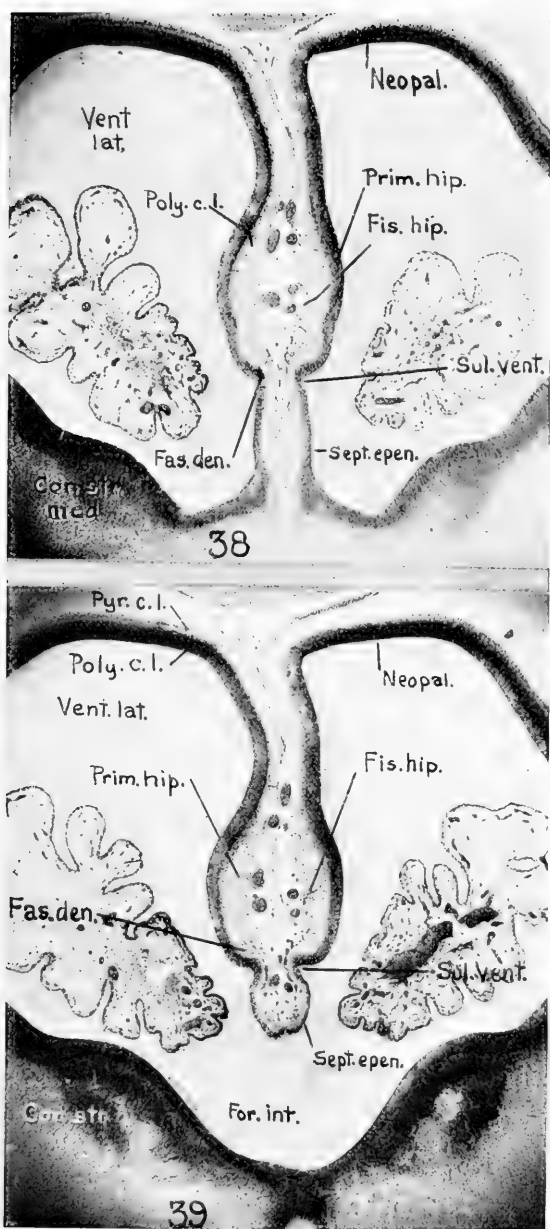
Fig. 33 Through the more rostral portion of the tuberculum olfactorium. This is comparable with the level shown of brain H 173, figure 29. The islands of Calleja appear as denser cell groups in the marginal velum of the ventral region.

Fig. 34 This section is slightly caudal to the previous one and illustrates the deepening of the embryonic fissura hippocampi.

Fig. 35 Through the septum ependymale. The marginal velum is just visible.

Fig. 36 Through the foramen interventriculare and the lateral choroid plexuses.

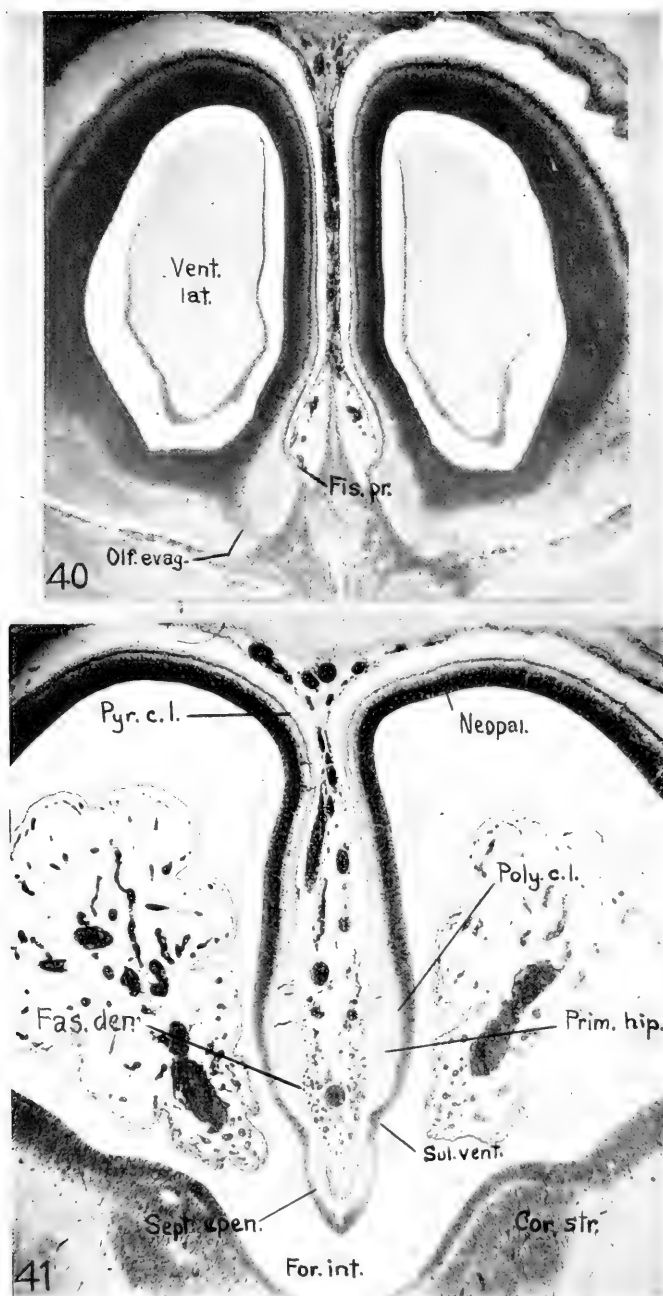
Fig. 37 Through the thalamus and the telencephalon caudal to the di-telencephalic groove. The roof of the third ventricle is non plexiform. The ependymal walls of both the lateral plexus and the diencephalic roof plate appear very thick. The tissue adjoining the taenia of the lateral plexus, both dorsal and ventral to it, is the hippocampus. The same type of cellular arrangement as that shown in the depth of the embryonic fissura hippocampi in more rostral levels is seen.



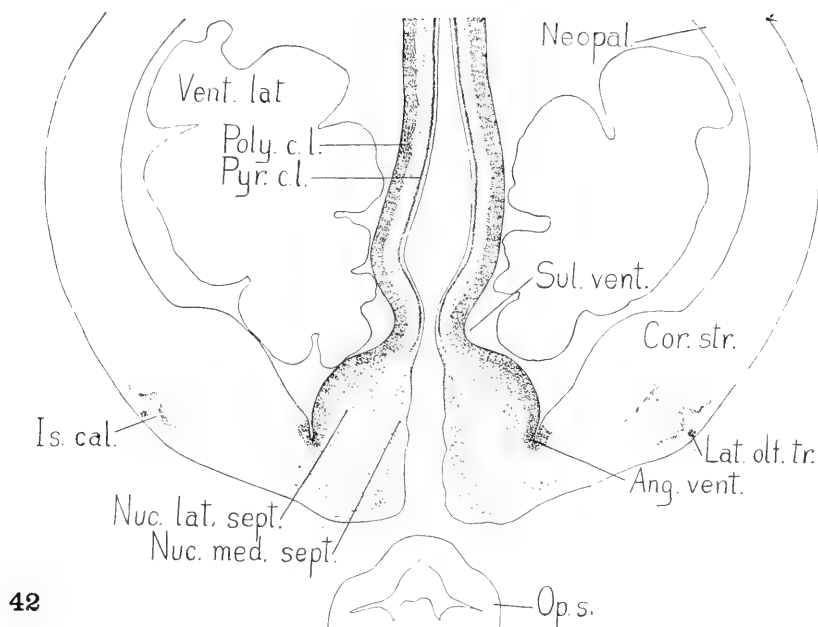
Figs. 38 and 39 These figures are reproductions of photographs of a 27.8-mm. embryo, belonging to the Chicago Collection. $\times 28$.

Fig. 38 Through the septum endymale and the lamina terminalis.

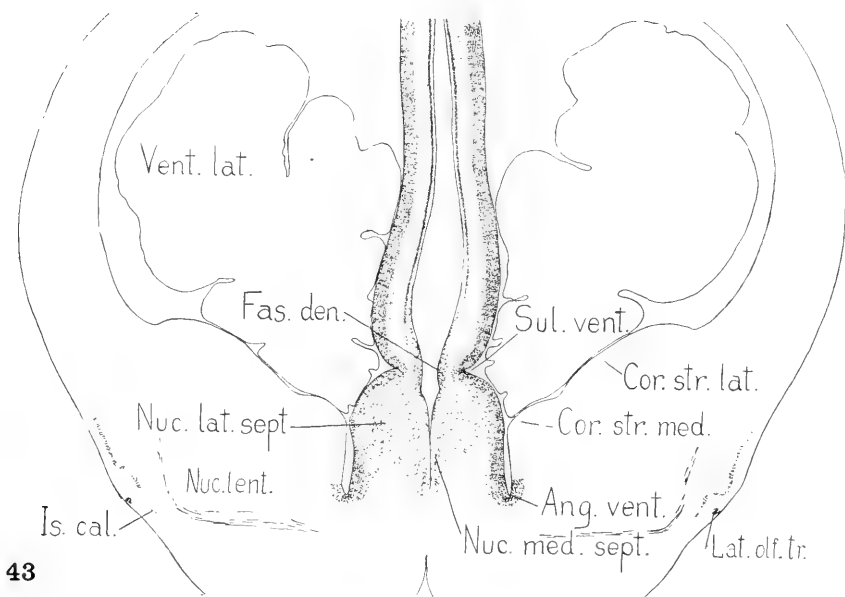
Fig. 39 Through the tela chorioidea telencephali medii and the foramen interventriculare.



Figs. 40 and 41 These photographs were taken from a transverse series of a human embryo 32.1 mm. in length, belonging to the Chicago Collection, H 41. $\times 28$.



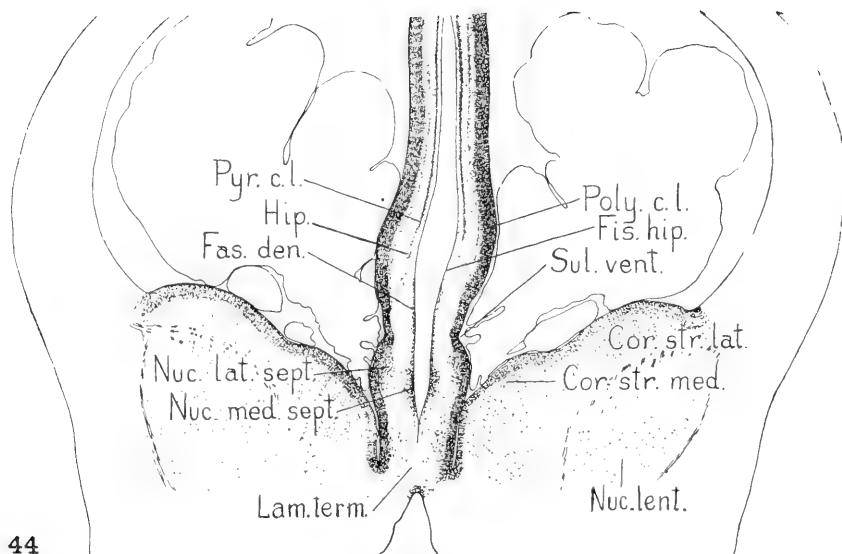
42



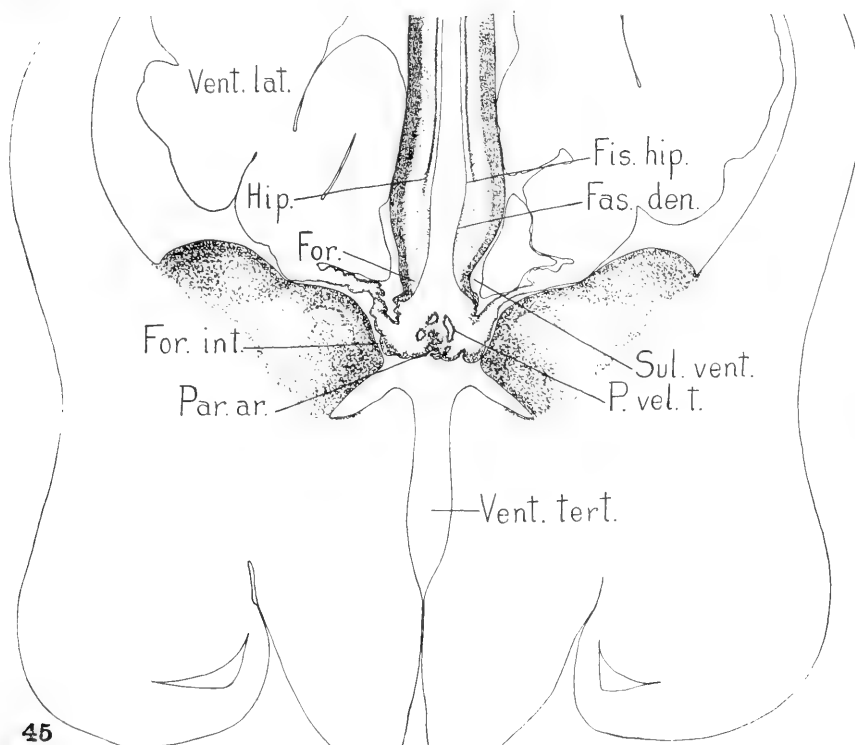
43

Fig. 40 Through the root of the bulbous olfactorius, showing the curve in the medial wall described by His as the fissura prima.

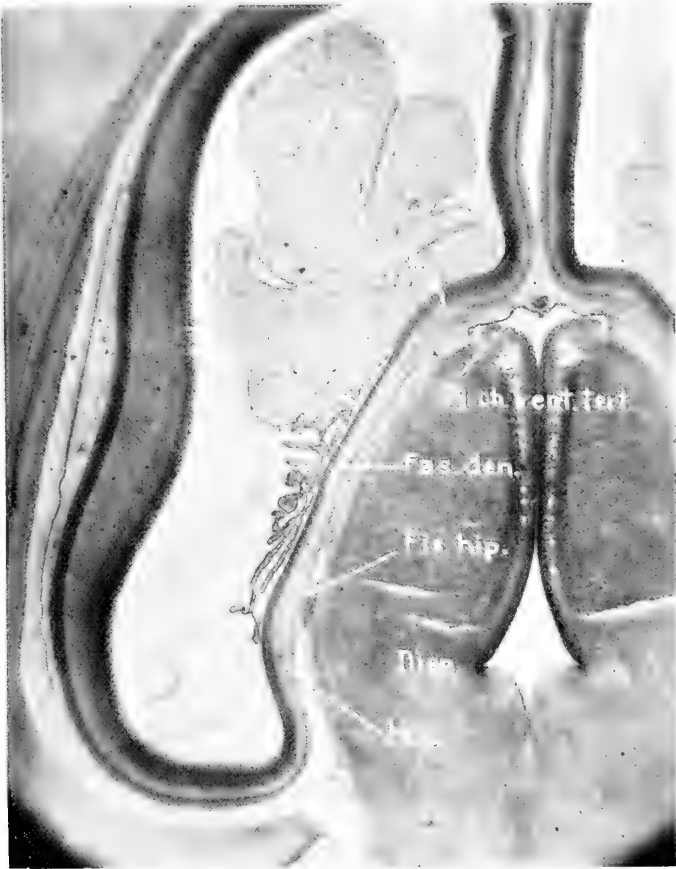
Fig. 41 Through the tela chorioidea telencephali medii. The primordial hippocampus is bounded by the sulcus limitans hippocampi ventrally, and by the neopallium dorsally. The lamination of the neopallial cortex is easily discernible. Ventral to the sulcus limitans hippocampi is the septum ependymale. Emerging from the matrix a narrow marginal velum is seen.



44



45



46

Figs. 42 to 45 Pen drawings of sections through the 39.1-mm. embryo, H 163. The planes of section are indicated on the drawing of the model, figure 18.

Fig. 42 Through the septum and the postoptic recess.

Fig. 43 Through the rostral portion of the lamina terminalis. The cortical lamination in the hippocampus reaches almost to the sulcus limitans hippocampi. The differentiation of the pyramidal cell layer seems tardier than that of the polymorphous layer.

Fig. 44 This section was taken through a more caudal part of the lamina terminalis. The slight groove in the medial wall, dorsal to a line joining both sulci limitantes hippocampi is the remnant of the fissura hippocampi of the earlier stages. The polymorphous and pyramidal cell layers are not well as differentiated in the regio hippocampi as they are in the two previous figures.

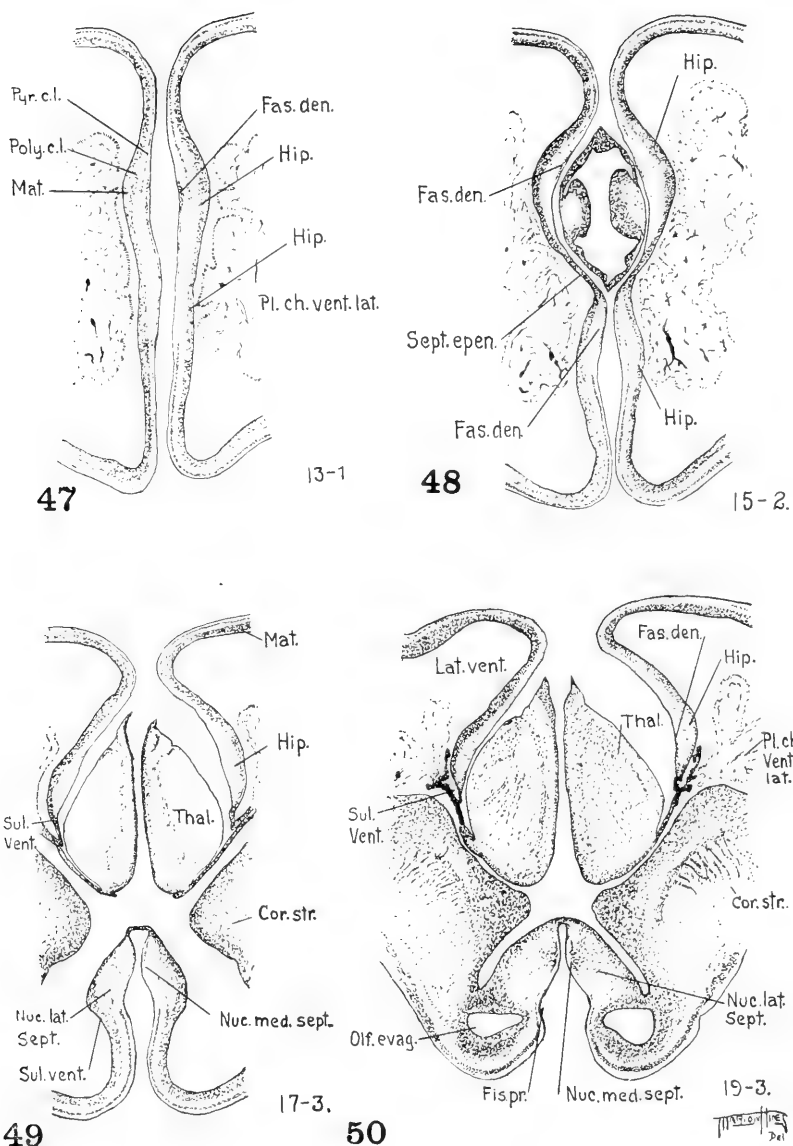


Fig. 45 Section through the parapyseal arch, showing a few postvelar tubules, the lateral choroid plexuses, and the foramen interventriculare. The cortical layers are not visible in the hippocampus, although they are well developed in the neopallium.

Fig. 46 Photograph from the same specimen as the last, through the depth of the hippocampal fissure. For plane of section see figure 18. A thin row of cells lies in the most medial part of the mantle zone of the hippocampus, fascia dentata. $\times 14$.

Fascia dentata

In the medial margin of the hemisphere wall in the 19.1-mm. embryo opposite the sulcus limitans hippocampi lies a group of cells, the fascia dentata. This group of cells does not appear in the younger embryos, but persists in the rest of the series as a mass of cells, which seem to have migrated out of the matrix lying opposite the dorsal limit of the sulcus. This differentiation begins anteriorly and passes posteriorly, following in development the initial differentiation of the future hippocampal region into the outer marginal velum and inner matrix layers. These cells slip along the marginal velum of the developing hippocampus. In the 39.1-mm. embryo and the 43-mm. they form a slender band almost coextensive on the medial wall ventro-caudally with the development of the hippocampal cortex. The rostral limit of the fascia dentata lies in close proximity to the rostral limit of the sulcus limitans hippocampi. The four sketches, in figure 51, present comparable levels through the region under discussion from four different embryos. A, a 16-mm. embryo, shows no differentiated fascia dentata, but in B, a 20-mm., a group of differentiated cells opposite the sulcus limitans hippocampi may be seen. In C, a 39.1-mm., these cells have slipped along the marginal velum of the hippocampus; while in D, an 85-mm., they show the characteristic crescentic line-up. In the levels delimited only that of the 85-mm. demonstrates the relation of the sulcus fimbrio-dentatus to the growing fascia dentata. Here the fornix fibers lie ventral to the dentate band, among undifferentiated cells of the primordium hippocampi, separated from that gyrus by the sulcus fimbrio-dentatus.

Figures 47 to 50 These drawings were made from a coronal series of the forebrain of a 43-mm. human embryo, no. 886, of the Mall Collection. Refer to figures 19 and 20 for the medial sagittal section of the model and the planes of these sections. $\times 6\frac{2}{3}$.

Fig. 47 Through the hippocampus, showing that tissue in both its caudal and rostral aspects.

Fig. 48 A section through the upper border of the thalamus, the septum ependymale, and the anterior and the posterior divisions of the hippocampus.

Fig. 49 Through the septal region and the posterior part of the hippocampus. Note the cellular groups in the septum.

Fig. 50 Through the caudal hippocampus, the middle of the thalamus and the olfactory bulb.

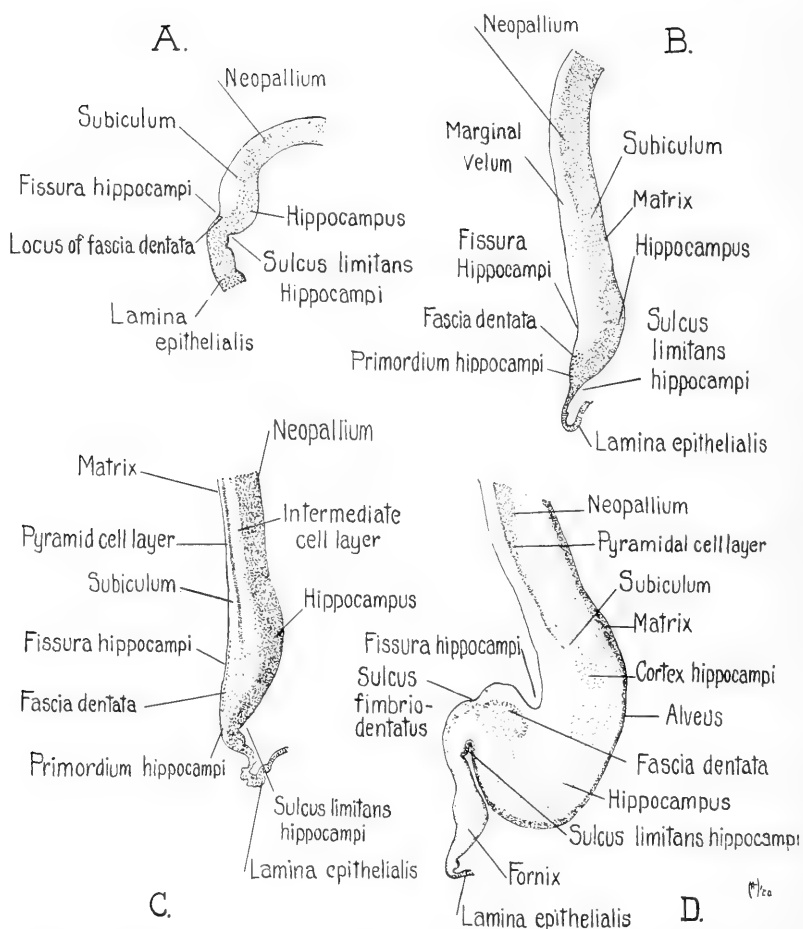


Fig. 51 These four pen-and-ink sketches were drawn either with the Edinger projection apparatus or were taken as tracings from photographs of known magnification. They show the relative histological development of the neopallium, the hippocampal formation, the fascia dentata and the lamina epithelialis, through a level which subtends the lateral choroid plexus itself or its primordium.

Sketch A. $\times 35\frac{2}{3}$. No. 465, 16 mm. (section 11-2-6), University of Chicago Collection. The hippocampal region is clearly delineated by the thickened marginal velum, the thin matrix, and the great convexity toward the ventricular surface. Ventral to the hippocampal formation lies the lamina epithelialis, the site of the lateral choroid plexus evagination. Here it is a thick wall made up of many small undifferentiated cells. The matrix of the neopallium interdigitates with the marginal velum. The fissura hippocampi is a shallow groove beneath which lies the locus of the future fascia dentata.

This last is essentially the reptilian condition, and, since some of these fornix fibers in both cases enter the alveus in the adult, it follows that the reptilian dorso-medial cortex is comparable with the human hippocampal cortex rather than with the fascia dentata, as supposed by Meyer ('92) and Levi ('94). Moreover, the embryological evidence presented by these embryos is decisive in favor of the same conclusion. The fascia dentata arises from cells of the matrix immediately dorsal to the sulcus limitans hippocampi, from which position its cells migrate dorsalward along the outer margin of the hippocampal formation. This mode of origin corresponds in all major particulars with

Sketch B. $\times 35\frac{5}{7}$. No. 460, 20 mm. (section 15-1-2), Carnegie Institution, Baltimore. The histological characters of the hippocampus are the same as those described for the 16-mm. embryo. The area itself is greater dorso-ventrally and the fissura hippocampi, although shallow, includes a greater sweep of tissue. The lamina epithelialis is thinner, composed of only a few rows of epithelial cells. Opposite the sulcus limitans hippocampi within the marginal velum lie a group of cells, the undifferentiated fascia dentata.

Sketch C. $\times 7\frac{5}{7}$. No. H 163, 39.1 mm. (section 147-1-4), University of Chicago Collection. The medial wall in the region of the hippocampal primordium bulges prominently into the ventricle, its ventricular convexity being greater than its medial concavity. Within the dorsal lip of the fissura hippocampi three cortical lamina are present: 1) the inner, or matrix; 2) the middle, or the intermediate cell layer, and, 3) the outer or row of young pyramids. The intermediate cell layer, like the matrix, is never as wide in the center or ventral lip of the fissure as it is in the dorsal lip or in the neopallium. The lamina epithelialis is more convoluted and presents at this level a concave surface outward, an indication, the writer believes, of the sulcus fimbrio-dentatus. The fascia dentata has migrated dorsally in the marginal velum of the hippocampus. The locus from which these cells come shows no cortical lamination, although between the fascia dentata and the matrix a few undifferentiated cells remain.

Sketch D. $\times 10\frac{5}{7}$. No. 1400-23, 85 mm. (section 34-1-4). A brain belonging to the private collection of Dr. George L. Streeter, studied at the Carnegie Laboratory of Embryology, Baltimore. The hippocampal formation lies in the depth of the fissure. The fascia dentata seems caught in a characteristic twirl. Ventral to the fascia dentata lies a small sulcus, the sulcus fimbrio-dentatus. The fornix fibers are intermingled with undifferentiated cells, the persistent primordium hippocampi. It takes no leap of fancy to bridge the growth process from this stage to the adult.

NOTE. In the untouched photographs from which figures 29-31, 33-36, 38, 39 and 41 were reproduced the histological differentiation of the fascia dentata was clearly visible, as indicated in the pen drawings, figures 42-45, 47-51. This detail in some of the photographs is lost in the reproduction.

Levi's ('04) description of the development of the fascia dentata in the rat, but his interpretation requires revision.

The identification of the reptilian dorso-medial cortex with the fascia dentata of Meyer and Levi has been questioned by Cajal ('11) and by Elliot Smith ('10), who believes, however, that it is undergoing differentiation toward fascia dentata, a view supported also by Crosby ('17) and in a modified form by Johnston ('13, p. 391, and '15, p. 419).

By what criterion shall the fascia dentata be known—nerve connections, intrinsic chromatin staining, position, or history? To determine nerve connections in this material is impossible. The primordial fascia dentata shows the characteristic intensive nuclear staining even in its earliest stages of development. But its morphological disposition can be so followed from stage to stage up to the adult form that there is no doubt as to its identity. In vertebrates below the lowest mammals there is no representative of this structure. Levi ('04) has pictured the dorso-medial cortex of reptiles as containing a cortical lamination of deeply staining cells, whose connections according to Smith and Cajal are those of the hippocampus. Its boundaries have nothing in common with those of the mammalian fascia dentata. But knowing the origin of this tissue in human development, we naturally turn to the homologous region in the lower vertebrates. Such an area in both reptiles and mammals is the undifferentiated primordium hippocampi in the region of the developing fornix fibers. If the fascia dentata is a center for cortical reinforcement, as Cajal ('11) thinks the neurone connections indicate, and not the main receiving station for incoming impulses over the medial olfactory tracts, as Elliot Smith ('96) maintains, then it would seem natural for its development to be in abeyance in lower vertebrates. But if the reverse be true, we are at a loss to supply a reason for its undifferentiated condition in lower forms. It seems logical that it may develop from the cells of the primordium hippocampi, opposite the sulcus limitans hippocampi, and that the development will be delayed in accordance with Cajal's hypothesis of its function, until cortical associational mechanisms are well elaborated. Be that as it may, its anatomi-

cal site of development supports the generalization of Elliot Smith ('96) for monotremes, that this tissue is the fringe of the cortex.

Hippocampus

Coincident with the appearance of the small area characterized by thicker marginal velum in the dorsal wall of the evaginating hemisphere in the 11.8-mm. embryo, there is a slight thickening of the wall itself. These two features furnish the first differentiation of the primordial hippocampus. The histological and morphological differentiation is more apparent in the 14-mm., and in the 19.1-mm. and the 20-mm. the whole extent of this tissue is involved in a groove, the fissura hippocampi. Here its wall is slightly thicker than the wall of any other part of the cortex. There is little cell migration from the matrix into the marginal velum. In fact, at this stage of development of the cerebral vesicle, this is the only region where a true cell-free margin is found. The tissue which lies immediately dorsal to the hippocampus is neopallium and that which is ventral is either septum or a derivative of the area epithelialis. This fissure, in all probability, is not formed by an invagination due to more rapid growth of the central part of this tissue, but rather by a buckling of the wall on itself as the result of the appositional growth of the neopallium between the endorhinal fissure and the dorsal limit of the hippocampus, plus perhaps the lack of support, except at one point, by the epithelial tissue ventral to it. The fissure is deeper and less broad where the ventral support is narrow. Since this fissure or groove lies above the sulcus limitans hippocampi and the fascia dentata and involves the whole wall containing the primitive hippocampus, there can be little doubt as to its identity. It is the fissura hippocampi or the fissura arcuata of His, the Bogenfurche of other authors. This fissure must not be confused with the sulcus limitans hippocampi or the sulcus fimbrio-dentatus. It corresponds to the fissura arcuata of Herrick ('10) in reptiles, Johnston ('13) to the contrary notwithstanding, if the evidence above be accepted, namely, that the definitive hippocampus is derived from the reptilian dorso-medial cortex.

In this case the ventral boundary of the hippocampal formation may be drawn by passing a plane through the wall at the level of the sulcus limitans hippocampi. Such a limit would correspond to one similarly drawn diagonally through the medial wall of the brain of *Phrynosoma cornutum* (Herrick, '10, fig. 61, p. 533) joining the sulcus limitans hippocampi and a groove on the ventricular surface just dorsal to nucleus lateralis septi. In the brain of the turtle (Johnston, '13, p. 391, and fig. 17, p. 435) a sulcus which lies above this hypothetical plane described by Herrick is called the sulcus fimbrio-dentatus. This is Herrick's *fissura arcuata*.

If, now, Johnston and others are correct in assuming that the mammalian fascia dentata is derived from the ventral part of the reptilian area of differentiated cortex above this so-called fimbrio-dentate sulcus, then the reptilian primordium hippocampi gives rise only to the mammalian fimbria and fornix bed and the term fimbrio-dentate sulcus is clearly appropriate, for this sulcus is defined by Johnston ('13, p. 391) as "lying between the fimbria and the developing fascia dentata." But, on the other hand, it has been shown in this contribution that the human fascia dentata actually is developed, not from the differentiated hippocampal cortex downward, but upward from the extreme ventral border of the primordium hippocampi. The hippocampal primordia of reptiles and of these human embryos are apparently strictly comparable structures. The fimbrio-dentate sulcus as defined by Johnston cannot, therefore, lie dorsal to the primordium hippocampi as he describes it.

We conclude, then, that there is no fimbrio-dentate sulcus either in reptiles or in the human embryos here described. The mode of its appearance in later developmental stages has not been determined in sufficient detail to enable the writer to treat the subject exhaustively, although sketch D in figure 51, taken from an embryo 85 mm. in length, clearly delineates the fact that the sulcus in question develops later than the fascia dentata and appears ventral to both the fascia dentata and the sulcus limitans hippocampi. It would appear to follow from the conclusion that if the reptilian *fissura arcuata* of Herrick's description (fimbrio-

dentate sulcus of Johnston) is represented in the human embryo at all, it must be homologous with the fissura arcuata of His and with the fissura hippocampi of the adult. If, however, this conclusion is adopted, it must be recognized that the fissure is very differently disposed with reference to the chief mass of the differentiated hippocampal cortex in adult reptiles and mammals. But the embryology of the region as far as followed in this paper is almost the exact duplication of the situation as described for reptiles by Herrick ('10, pp. 464, 465). And the writer is inclined to think that future study will prove those differences discussed by Herrick to be slight indeed, for the fascia dentata arises from cells in the ventral lip of the fissura, immediately opposite or slightly dorsal to the ventricular sulcus limitans hippocampi. The fibers of the fornix lie between the matrix and the fascia dentata in this region. Moreover, the ventral lip of the fissura hippocampi shows no cortical lamination in the stages presented. This differentiation of the early hippocampus into a dorsal cortical portion and a ventral non-cortical resembles the reptilian condition, with the exception that opposite the sulcus limitans hippocampi lies the undifferentiated fascia dentata. There is nothing, according to Johnston or Crosby which compares to this differentiation of fascia dentata in either the turtle or the alligator. The writer suggests that the regions in adult reptiles called by these authors *primordium hippocampi* are the source of the fascia dentata.

There are some points, essential for the completion of this argument which remain obscure. In the material available it is impossible to determine the absolute ventral limit of the *primordium hippocampi* caudal to the *angulus terminalis*.

The *paraphysis* is universally regarded as a differentiation within the roof plate. The *lamina epithelialis* in all probability should not be so regarded since it takes part in the evagination of the hemisphere. Its subsequent position, however, is not evident until the 14-mm. embryo is studied. The *sulcus limitans hippocampi* in this contribution is regarded as marking the ventral boundary of the hippocampal formation. In the region rostral to the *angulus terminalis* (figs. 3 to 6, sketches 1 v) it

separates cortical from subcortical regions; but throughout the regions bordering the post-terminal area epithelialis it separates the thin area intercalata and (in the adult) the choroid fissure from the hippocampal formation. In the series presented no cortical areas are found ventral to it, nor are there any cells which can be proved to be neuroblasts ventral to it. But in figure 51, sketch D, it seems impossible to determine just where the boundary between the primordium hippocampi and the lamina epithelialis should be drawn. In the sketches it has been assumed to lie as indicated, below the area containing undifferentiated cells of the primordium hippocampi. On this interpretation that portion of the fimbria marked *Fornix* (fig. 51, D) is a segment of the lamina epithelialis which has been secondarily thickened by the invasion of fornix fibers; but possibly, it should be regarded as belonging within the primordium hippocampi, a possibility which cannot be disregarded until the methods of neurological technique have demonstrated otherwise. Doctor Herrick suggests to me that a close series of developmental stages of this region in reptiles or lower mammals would probably be favorable material for the solution of this question.

Fissura hippocampi

Having established the homology of the fissura hippocampi with the reptilian fissura arcuata of Herrick, we are desirous of clearing the situation as it exists in the history of the embryology of this region. As development proceeds, the primitive hippocampus presents a smooth contour from its earliest definition (9 mm.) to 16 mm. From that length to approximately 24 mm. the fissure extends into the medial wall from the base of the olfactory evagination to the end of the hippocampal primordium as a shallow groove involving the whole of this peculiarly differentiated area. For approximately the next 10 mm. of growth in greatest length, the fissure grows progressively shallow in the region above the area chorioidea, so that from the surface it appears to be divided into two segments. However, there is no interruption of the hippocampal formation itself.

During this time of development a new groove appears on the medial wall, the result of active olfactory bulb evagination, the fissura prima of His. The 27.8-mm. and the 32.1-mm. belong to this group. In the 39.1-mm. and the 43-mm. the anterior segment of the fissura hippocampi has disappeared, but the posterior and the fissura prima persist. The cortical lamination of the dorsal lip of the fissura hippocampi is coincident with the flattening of the medial wall.

If a dorsal commissure were added to the anterior commissure, now lying in the much-thickened lamina terminalis, the relationships of commissure, fissura hippocampi, and fascia dentata would resemble those of the marsupial. Elliot Smith ('97, p. 67) wrote of this comparison as follows:

In the Marsupial we have a *fissura arcuata* or *hippocampi*, extending from the tip of the temporal pole right round the mesial wall of the hemisphere towards the olfactory peduncle; so, in the fetal child or kitten, we find the Bogenfurche (which we might, with Mihalkovics, appropriately call 'Ammonsfurche') following a similar course and shading away towards the cephalic pole of the hemisphere. And it is necessary to remark, in passing, that the so-called part of the 'Vordere Bogenfurche,' which His calls 'fissura prima' has nothing whatever to do with the true Bogenfurche or fissura arcuata, if we regard the latter as the primitive fissura hippocampi.

Smith refers to the 1891 paper of Marchand. Moreover, Marchand ('09) denied the existence of such a fissure and Smith ('03) reports that Hochstetter's work on fissuration of the medial wall proves beyond a doubt that all the fissures are artefacts. But the conditions of these tissues in the brains of the 39.1-mm. and the 43-mm. are essentially the same as described by Smith in his first paper. His himself ('04) states plainly that the fissura prima has no relation to the fissura arcuata or the hinterer Bogenfurche; he defines it as follows: "The continuation of the fissura mesorhinica extends for a distance over upon the medial wall of the hemisphere as the fissura prima. By a deepening of the surrounding sulcus the termination of the lobus olfactorius or this bulbous portion becomes separated more and more from the overhanging frontal lobe. The bulbous portion retains its sagittal direction and becomes separated laterally from a

transversely directed portion by a deep sulcus" (p. 66). This fissure is considered by His to be the same as the 'vordere Bogenfurche.' The first embryo to have such a fissure is Se (16 mm. G. L.) thought by His to be six weeks of age. There is little doubt that this measurement in no way compares with those in this series. Here there is no well-developed olfactory bulb until the 27.8-mm. is examined, although there is a slight olfactory evagination in the 19.1-mm. Then the fissure in all of the series after 27.8 mm. is the same as that of His' description. It is quite possible that the bulb in His' embryos was delimited artificially. His also models a small bulb in C. R. (13.6 mm.), in which case the writer believes that some of the olfactory fila may have been included in the drawings of the projection of the brain. In the later stages the anatomy of the fissure is the same in all cases. Further, he thinks it divides the olfactory system into two parts. "At no time does it extend posterior to the lamina terminalis. Its remnant is the fissura parolfactoria posterior of the B. N. A." (His, '04, p. 76). This fissure is not, then, the anterior portion of the fissura arcuata; rather it is continuous with the mesorhinc fissure.

Besides this fissure in embryos of the second month, His finds a sickle-like fissure extending posteriorly beginning in the region of the terminal plate. He finds also that in many cases the mesenchyme fills the fissure and that there are no evident postmortem artefacts. He finds the same kind of thickening in the medial wall in the cat embryos of 14 mm. G. L., as Zuckerkandl ('01) showed in his paper on the development of the corpus callosum. The hintere Bogenfurche lies dorsal to the fissure of the choroid plexus, its anterior limit does not pass beyond the terminal plate. This fissure is undoubtedly the one the writer has identified as the fissura hippocampi. However, besides these, His described another, the 'accessorische Bogenfurche,' in his drawings of three- and four-month embryos. This lies on the anterior part of the medial wall, arching over the terminal plate. The writer finds nothing to correspond with this fissure and considers it an artefact.

Martin ('94), on the other hand, uses *vordere Bogenfurche* as synonymous with *fissura prima* and reports that it appears dorsal to the choroid fissure. He thinks also that the *hintere Bogenfurche*, a groove in the medial wall in the midst of the posterior hippocampus, does not join the anterior *Bogenfurche* until later in development. If his illustrations are carefully studied, it appears that Martin's *vordere Bogenfurche* is the anterior limb of the hippocampal fissure and seems to become continuous with the *hintere Bogenfurche* when the tip of the temporal pole has grown ventrally and rostrally. This finding, if so interpreted, agrees in all points with mine. There can be no doubt that Martin's *vordere Bogenfurche* and His' *fissura prima* are not the same. Grönberg ('01), however, found no separation of the *Bogenfurche* into anterior and posterior limbs in the hedgehog. This finding of Grönberg agrees in all points with that in man, namely, a fissure coextensive with the primordium of the hippocampus upon the medial wall.

Such workers as Hochstetter, Retzius, Goldstein, and Symington report that in the region of the primordium hippocampi there is a slight thickening of the wall, but no definite infolding, although upon careful examination the medial wall at this point is not smooth. In other words, these fundamental findings agree with those presented in this paper. Investigators who concerned themselves with well-fixed brains of the third and fourth month did not find the radial folds of the earlier work, nor could they identify the *fissura arcuata* of His. The confusion arose out of failure to distinguish between artefacts of fixation and the accentuation of normal findings. There is no doubt but that maceration plays havoc with the normal contour of the medial wall of the hemisphere before the fibers of the corpus callosum have lent their stiffness toward its support. His failed to emphasize the histological structure which he found in the *fissura arcuata* as peculiarly distinguishing that fissure from the accessory fissure above.

My special contribution to this particular phase of the investigation is the discovery that at a certain stage in development, the *fissura hippocampi* is coextensive with the hippocampal

primordium and that, as cortical differentiation proceeds in that portion which lies anterior to the velum transversum, the hippocampal fissure disappears. But posterior to the velum transversum the fissura remains as the adult fissura hippocampi (fig. 51, sketch D).

The relation of the hippocampus to the neopallium

The tissue which manifests the most marked and most regular acceleration is the neopallium. In the youngest embryo there is no clear line of lateral demarcation of this tissue. But in the 14-mm. the wall of the ventro-lateral sector is noticeably much thickened. This division between the two lateral sectors is more marked in the 19.1-mm., where a slight ventricular groove appears. The position of the hippocampus in the developing vesicle depends largely upon the amount of neopallium joining the hippocampus and the latero-ventral complex. Further, the intrinsic differentiation of the neopallium which is first seen in the 27.8-mm. progresses more rapidly than that of the hippocampus, although that tissue was the first to become at all evident in the developing telencephalon. The process of evagination is largely one of growth between the hippocampus and the region of the pyriform lobe, uncus and tail of the caudate nucleus.

The appended table 5 gives an idea of the relative differentiation in the vesicle of these various embryos.

With these data in hand two factors appear to be involved in the position of the developing archipallium. The first of these is the disposition of the old cortex in the wall of evagination, coincident with the noteworthy acceleration of the neopallium. The second factor is the intrinsic differentiation of the hippocampus itself. From these relationships it is possible to delineate the method of growth in the evaginating telencephalon.

Recalling the form of the telencephalon at a stage where no cortical area has been evaginated from the telencephalon medium into the cerebral hemisphere as exemplified by the case of *Ichthyomyzon concolor* already cited (Herrick and Obenchain, '13, figs. 3 and 4), it is evident that in the process of further

evagination the most dorsal edge of the massive side wall will become the most ventral edge of the complete evagination and help form the roof of the foramen Monroi (p. 126). Anterior to the lamina terminalis the most dorsal border will meet the most ventral edge, making a seam or junction along the medial wall of the growing telencephalon.

This seam or junctional zone on the medial wall of the cerebral hemisphere in front of the lamina terminalis is always marked in amphibian and reptilian brains by a cell-free limiting zone and often by a ventricular groove, a sulcus limitans hippocampi. This area is necessarily transitional in type because, besides the approximation of the hippocampal formation with the septum (the primitive dorsal column with the primitive ventral column), it marks the union of the thinner dorsal part of the telencephalic roof plate bordering the hippocampal formation with the septum, the major portion of which sooner or later becomes greatly thickened. The sulcus limitans hippocampi marks the border of the hippocampal formation for its entire length, and rostral to the foramen interventriculare it also marks the junction of the hippocampal formation with the septal complex.

In this series the cerebral hemispheres of the 11.8-mm. embryo have reached what may be called the first stage in the evagination; the primordial hippocampus is dorsal throughout and there is no medial wall anterior to the lamina terminalis. The hippocampal formation lies as a crescent over the top of this evagination, never quite reaching either the anterior or the posterior pole of the vesicle, yet lateral and dorsal to the sulcus limitans hippocampi. In the 14-mm. the hippocampus is entirely medial, anterior to the angulus terminalis, and climbs, as it were, over the crest of the hemisphere to the posterior pole. Here again the differentiation only approaches the posterior pole, but does not reach it. The amount of neopallium is greater in this embryo at the anterior pole than at the posterior. This process continues, so that the formation in the 19.1-mm. and the 20-mm. lies entirely upon the medial wall. In the latter embryo, however, the neopallial tissue in the posterior pole has increased. In the 27.8-mm. and the 32.1-mm. the neopallium has grown greatly

Summary of development of

EMBRYO		TUBERCULUM OLFACTORIUM; OLFACTORY BULB	SEPTUM	AREA CHORIOIDEA			
Num- ber	Length			Septum ependymale	Area inter- calata	Lamina epithelialis	Corpus striatum
	<i>mm.</i>						
1121	11.8	Olfactory fila only	Thick wall	Thin wall	Thin wall	Thin wall	Ventro-lateral sector thicker
940	14.0	Olfactory fila + a slight evagination	Same	Same	Same	Concave out- ward	Angulus ventralis ap- pears; ventro-later- al sector much thicker
H173	19.1	Same with slightly more evagination; cortex of tubercu- lum olfactorium just visible	Nucleus medialis septi	Same	Same	Lateral cho- roid plexus	Two hillocks and a few fibers in the lateral gray
460	20.0	Same; cortex of tu- berculum more prominent	Same plus margi- nal velum	Same	Same	Same	Same
H91	27.8	True olfactory bulb and fissura prima; same	Same	Same + mar- ginal velum	Same	Posterior limb of lateral cho- roid plexus greater in extent	Marked increase in cells lying between the caudate nucleus and marginal ve- lum
H41	32.1	Same + differentia- tion of layers of bulb	Beginning of nu- cleus lateralis septi	Increase in marginal velum, whole area thicker	Same	Same	Same plus few inter- nal capsule fibers
H163	39.1	Same; pronounced islands of Calleja	Large nucleus lat- eralis septi + nucleus accum- bens, anterior commissure	Wide margi- nal velum	Same	Same	Nucleus lentiformis, internal and exter- nal capsule fibers
886	43.0	Same	Same	Same	Same	Same	Same

LE 5

parts of the cerebral hemisphere

EMBRYO		PYRIFORM LOBE	HIPPOCAMPUS	FASCIA DENTATA	NEOPALLIUM
Num- ber	Length <i>mm.</i>				
1121	11.8	Ventro-lateral sector thicker	Narrow marginal velum; sulcus limitans hippocampi; ventricular ridge	None	No differentiation
940	14.0	Same	Occupies medio-dorsal wall of hemisphere, wider marginal velum	None	Marginal velum contains neuroblasts
1173	19.1	Appearance of sulcus on ventricular wall between neopallium and corpus striatum	Same plus a few cells in the marginal velum. Fissura hippocampi	Few cells opposite the sulcus limitans hippocampi	Neopallial tissue appears on the medial wall
460	20.0	Same	Same	Same	Appearance of neopallial tissue posterior to hippocampus
H91	27.8	Two definite cell layers on contour of these nuclei	Same	Same	Increase in total tissue; cortical layers
H41	32.1	Same	Two cortical lamina in dorsal lip of fissure	Same	More tissue
H163	39.1	Appearance of lateral nucleus and tract; cortical layers; endorhinal and ectorhinal fissure	Intermediate cell layer in ventral lip	Growth dorsally along marginal velum of hippocampi	Same
886	43	Same	Same	Same	Same

in the posterior pole and the hippocampus has made the ventro-caudal twist into the temporal lobe so characteristic of it. In the last two embryos of the series, the 39.1-mm. and 43-mm., remarkable growth has taken place in all regions of the neopallium, so that relatively little area, comparatively speaking, contains the hippocampal formation. And it is worth noting that the major portion of the hippocampus in these last two brains lies in the medial wall posterior to the velum transversum. Thus tracing the history of its position in the developing hemisphere lends adequate support to a portion, at least, of Herrick's quadrant theory of telencephalic evagination. But, further, this brief history of hippocampal position points to the conclusion that its extent is inversely proportional to neopallial growth. It also gives some facts concerning the regional acceleration of this neopallial growth, namely, that acceleration seems to shift from the frontal to the dorsal and then to the posterior poles of the developing hemisphere.

Concomitant with this change there is the intrinsic differentiation characteristic of the hippocampus itself. Although set aside as the first cortical area, its subsequent differentiation progresses so slowly that such layers as are characteristic of the cortex appear in the neopallium long before they are completed in the hippocampus.

The differentiation does not proceed in any logical sequence, but seems rather to be subject to rhythms of acceleration. These rhythms of acceleration do not correspond absolutely to those expressed in any arrangement of the adult brains of the vertebrate phylum. In other words, given the stage in human development of the hippocampus, the differentiation of the fascia dentata or the neopallium will not correspond to the phylogenetic development of the first-named tissue. It is possible, however, to take any one tissue and follow it through a complete development whose changes fit into its phylogeny. The developing neopallium seems to act as a disturbing factor, not, however, as one which obliterates, but rather as one which obscures the phylogenetic history by suddenly leaping into the foreground and by its great increase in amount and complexity of tissue

demanding immediate and engrossing attention. It is a disturber of growth rhythms and an obscurer of elementary phylogenetic 'patterns.' It is the belief of the writer that there is actually some relationship between these two; that is, that the acceleration of the neopallium results in a change of rhythm of growth in different parts, although it has no effect upon the actual differentiation, except that of obscuring it.

SUMMARY

1. The medial wall of the cerebral hemisphere of human embryos 16 mm. to 30 mm. in length is not 'perfectly smooth.' Its otherwise even contour is broken by a shallow groove. This groove extends from the region of the olfactory bulb to the tip of the temporal pole. It is the fissura hippocampi, the 'Bogenfurche' of His. It is homologous with the fissura arcuata of reptiles as described by Herrick.

2. In embryos as young as 11 mm. the primordial hippocampus can be recognized along the line of the future fissura hippocampi. This primordium is identified by the following histological peculiarities: 1) a thicker wall; 2) a narrower matrix; 3) a clearly defined marginal velum; 4) a limiting sulcus, the sulcus limitans hippocampi. This is the first cortical differentiation known in man.

3. The fascia dentata arises in the matrix of the hippocampal formation from cells in the dorsal limb of the sulcus limitans hippocampi. These cells grow dorsalward, slipping along the marginal velum of the hippocampus. In the series studied no other cortical differentiation has occurred in this region. It is comparable to the persistent primordium hippocampi of amphibians and reptiles.

4. The fissura prima of His first appears in embryos of about 25 mm. The appearance is coincident with the marked evagination of the olfactory bulb. It has no connection with either the fissura hippocampi or the hippocampal primordium.

5. The various regions of the telencephalon medium are distinguished by a characteristic morphology and histology in all the embryos of this series except the 11.8-mm. In the

remainder of the group described the angulus terminalis separates the midline structures into terminal plate and roof. The former is the lamina terminalis; the latter, the area chorioidea. The lamina terminalis increases in length and width throughout the series. The area chorioidea changes little in total length. Its anterior division, the tela chorioidea telencephali medii, is practically stationary. Its posterior division, the parapyseal arch, in the younger embryos forms a tent-like evagination in the roof; in older stages it may become a pouch-like paraphysis with two lateral pockets.

6. The portion of the medial wall of the hemisphere contiguous with the area chorioidea and the dorsal thin part (*pars tenuis*) of the lamina terminalis is termed the area epithelialis. It may be divided into the following parts, enumerated from ventral to dorsal borders:

1) The septum endymale (fig. 14, *Sept. epen.*) is that portion of the area epithelialis which lies ventrally of the angulus terminalis and borders the dorsal thin portion of the lamina terminalis. In later stages it thickens, beginning at the ventral border, differentiating first into matrix and marginal velum, with later migration of neuroblasts of the septal nuclei into the latter. The dorsal portion remains thin and undifferentiated.

2) The area intercalata (figs. 14, 16, *A. int.*) lies contiguous to the tela chorioidea telencephali medii. It remains membranous and increases but slightly in total surface and thickness.

3) The lamina epithelialis (figs. 14, 16, *Lam. ep.*) borders the parapyseal arch and becomes transformed into the lamina epithelialis of the lateral choroid plexus of the adult. Its anterior moiety subtends the parapyseal arch and the invagination of the choroid fissure begins between the 14-mm. and the 16-mm. stages. Its posterior moiety contiguous to the di-telencephalic fold of the velum transversum thereafter rapidly expands.

7. The neopallium grows more rapidly than any other part of the telencephalon. Its initial differentiation follows that of the hippocampus; its subsequent development surpasses that of the latter. The identification of the future hippocampus in the

young stages suggests a certain type of relative growth in the telencephalon. In measuring the growth of the histologically distinct regions of the telencephalon medium and the areas contiguous to them, lying in the evaginated portion of the hemisphere, we are able not only to measure the relative amount of growth of the telencephalon, but also to determine the manner in which this growth takes place.

In the embryos studied the medial wall was observed to grow in the following manner: first, by the intrinsic growth in the midline, especially in the region of the lamina terminalis and in that of the di-telencephalic fold; second, by the out-growth of a series of arcs of new tissue which forms the incipient frontal-parietal, occipital, and temporal poles.

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Resumen por el autor, N. E. McIndoo.

El sentido del oído en la abeja.

Órgano productor del sonido: Este aparato consiste en membranas situadas entre las axilares en las bases de las alas anteriores. Los músculos del tórax que se insertan sobre estas axilares se contraen y dilatan con rapidez, produciendo de este modo una vibración de aquellas; a consecuencia de esto las membranas vibran rápidamente, produciendo el ruido semejante a un chilido que se percibe al comprimir una abeja. Los experimentos llevado a cabo por el autor no prueban que las abejas pueden oír. El autor cree que las abejas no oyen, por lo menos del modo que nosotros oímos; pero a juzgar por todas las pruebas experimentales y anatómicas que poseemos, parece que su sentido del oído no puede separarse de su agudo sentido del tacto, del mismo modo que su sentido del gusto no puede separarse del del olfato.

Los supuestos órganos del oído: Hasta el presente se han hallado cinco llamados órganos auditivos en la abeja. A juzgar por su anatomía, las placas porosas, los frascos de Forel, las excavaciones provistas de una prolongación obtusa y el órgano de Johnston, todos ellos situados en las antenas, no parecen bien equipados para actuar como receptores del sonido; pero los órganos cordotonaes, situados en las tibias, pueden estar mejor adaptados para esta propósito, si es que tienen una porción externa correspondiente al tímpano. Las placas porosas pueden ser sensitivas a las corrientes débiles de aire y tal vez funcionen como un aparato de presión de aire que sirve para informar a las abejas de un objeto situado inmediatamente delante de ellas. Los órganos de Johnston pueden ser órganos estáticos que sirven para registrar los movimientos del flagelo. Las funciones de los frascos de Forel, excavaciones con prolongación obtusa y órganos cordotonaes son problemáticas.

THE AUDITORY SENSE OF THE HONEY-BEE

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TWENTY-SIX FIGURES

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INTRODUCTION AND METHODS

Much has been written about the auditory sense of insects, but critics still contend that it has never been demonstrated beyond a doubt that any insect can really hear. Most students on insect behavior believe that insects can hear, but only Turner and Schwarz ('14) and Turner ('14) seem to have produced good experimental evidence; however, they used only moths in their work. Much less is known about the sound perceptors in insects, and still it is not generally known how insects make sounds which are supposed to be heard by them.

It is usually believed that insects can hear for the three following reasons: 1) many have special sound-producing organs; 2) some have so-called auditory organs, and, 3) many of the experimental results obtained indicate that insects can hear.

In regard to the buzzing of insects, there are four old views to explain how the noise is made, as follows: 1) by the rapid vibration of the wings; 2) by the vibration of the thorax; 3) by a special modification of the oclusor apparatus in the stigmata, and, 4) in Diptera, by the vibration of the halteres. Relative to Diptera and Hymenoptera, Pemberton ('11) and Aubin ('14) show that none of the above views hold good. They determined that the buzzing is made by the extreme bases of the wings, as is shown for the honey-bee in the present paper. The humming, or more common noise produced by the wings, is made by the distal portions of the wings.

We should not expect insects to respond to sounds which have no significance to them, nor to sounds not in their category, because they may not hear the sounds that we do. The number of vibrations perceptible to the average human ear varies from 32 to 60,000 per second. Now it may be that the insect ear is so poorly developed that it can hear only sounds having vibrations below 32 per second. It may also be that the sense of hearing in insects is on no higher plane than that advocated by Forel ('08), who believes that insects do not hear, at least as we do, but compares this perception in them to that in deaf-mutes who feel the rolling of a carriage at a distance.

Bee-keepers are agreed that bees can hear, yet they cannot prove it. Von Buttel-Reepen ('07), a scientist and an experienced bee-keeper, in discussing the behavior of bees has much to say about their auditory perception, but still he produces no experimental evidence to support his strong statements.

To obtain material for the structure of the sound-producing organ, adult bees were used; but for a study of the so-called auditory organs, young bees, nineteen and twenty-one days old (counting from the time the eggs were laid) were employed. Fresh material was fixed in the modified Carnoy's fluid and was embedded in 60° paraffin. Sections were cut 5 and 8 μ in thickness, and were usually stained in Ehrlich's hematoxylin and eosin, but a few of them in eosin alone. All the drawings are original and were made by the writer at the base of the microscope usually with the aid of a camera lucida.

SO-CALLED VOCAL ORGANS OF INSECTS

In the higher animals the vocal organs are located in the throat, but in insects we should not expect to find their vocal organs in the buccal cavity, because this class of animals has a totally different organization. Hence, we must look elsewhere for the vocal organs of insects.

1. Sound-producing organ of honey-bee

In 1911, while determining that bees have an olfactory sense, the writer also ascertained that they have a means of making a piping or squealing noise.

a. Experiments to determine how bees make sounds. Hundreds of worker bees with wings pulled off or cut off were tested, and from mere observations it appeared that the squealing noise was made by the thorax. To be sure that the noise was made by the thorax, the abdomens of several bees were cut off. These mutilated bees could walk fairly well and when irritated made the squealing noise. Several other bees with both heads and abdomens cut off were likewise tested; these were so badly mutilated that in only one case did a thorax make the noise. Bees with wings cut off, when observed under a binocular, further confirmed the view that the squealing noise is made solely by the thorax, because the bases of the wings were seen to vibrate rapidly.

In 1920 the preceding line of experimentation was continued, and the following results were obtained. The loud buzzing of bees is made by the distal portions of both pairs of wings, while the squealing noise is made only by the bases of the front wings. When worker bees or drones were squeezed, or when their wings were held firmly, or were cut off or pulled off, these insects usually made the squealing noise. When no noise was heard, the insect being tested was placed on a large inverted pan lying on a table. The pan thus serving as a resonator usually intensified the feeble noise so that the human ear could hear it. Bees with the wing stubs glued did not make the noise, but after dissolving off the glue the insects made the noise as usual. When the muscles attached to the roots or axillaries of the wings were cut, the

squealing noise ceased for all time, and when the thorax of a live bee was held gently between the fingers the tingling sensation perceived indicated that these muscles vibrate very rapidly, setting in motion the axillaries and membranes in the bases of the wings. A microscopical examination of all the front wings pulled off showed that every bee with wings thus detached was able to squeal so long as one or two intact axillaries remained in the thorax.

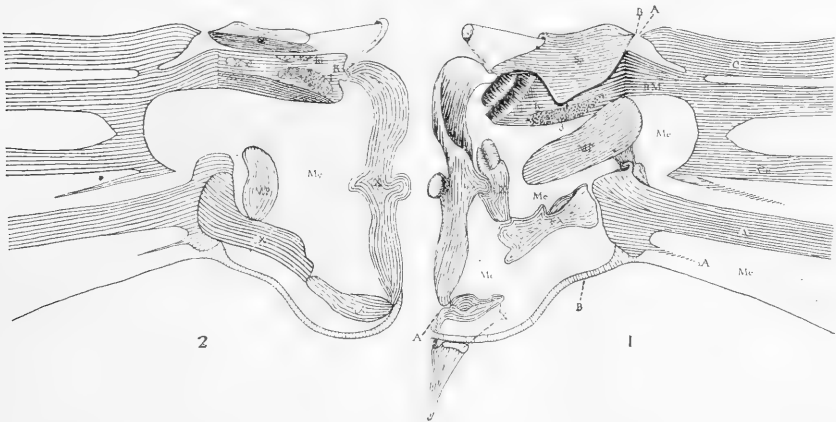
Besides the buzzing and squealing noises made by bees, the writer often heard a crackling sound while observing these insects flying around an alighting-board. He could not detect how this sound is made, but imagined it produced by the wings striking together accidentally.

All attempts, except one, trying to get bees to respond to the squealing of other bees failed. Or at least the bees exhibited no reactions which could be attributed as signs of hearing. Nevertheless, one squealing bee was held in a hidden position a few inches from an alighting-board; at once one of the many workers on this board seemed to take notice and flew to the screen behind which the squealing bee was hidden, and then it came immediately to the squealing bee, which it began to examine by running around it and smoothing its hair.

A queen bee, resting on a comb with workers surrounding her, when squeezed, squealed and the near-by workers became excited. Such experiments really do not mean much, because too many interfering factors cannot be eliminated. The original plan of the writer was to carry on experiments in which he hoped to be able to classify and to record on phonograph records the various sounds heard in a hive of bees. If this were possible, he intended to reproduce these sounds and then to determine whether or not bees respond to them. When he was transferred from the division of Bee Culture, this line of experimentation was discontinued.

b. Morphology of sound-producing organ. Several live worker bees and drones were held under a binocular and the following observations were recorded: When a bee is held by the legs it buzzes continuously. The wings are held straight out at right

angles; their anterior margins move little, but their flexible posterior portions vibrate rapidly; their bases do not vibrate, but move slowly in and out and backward and forward; no squealing was heard, but the muscles in the thorax vibrate more or less slowly. When one-half of each wing was cut off, a faint buzzing and a feeble squealing noise were heard. When the front wings were cut off as closely as possible and the hind wings were pulled out by the roots, no buzzing was heard, but the squealing noise was quite pronounced. While the bases of the front wings



Figs. 1 and 2 Base of right front wing of a worker honey-bee, showing special sound-producing apparatus, consisting of membranes (*Me*) lying between axillaries (1, 2, 3, and 4*X*), median plate (*MP*), head of radius (*R*), subcosta (*Sc*), costa (*C*), union of radius and media (*RM*), cubitus (*Cu*), and anal veins (1 and 3*A*). Nos. 1, 1*a*, 2, and 3, groups of olfactory pores. Fig. 1, dorsal view, and fig. 2, ventral view. $\times 40$.

vibrated, two weak lines in them were exhibited, as indicated by lines *AA* and *BB* in figure 1. The first one, starting between the ends of the costa (*C*) and subcosta (*Sc*) and ending between the first (1*X*) and fourth axillaries (4*X*), resembles a stiff hinge; while the second one, starting from the same source, passes through the weak point in the union of the radius and media (*RM*) and ends between the first anal (1*A*) and third axillary (3*X*). This line, along which the wing usually breaks when this appendage is carelessly pulled off, is more rigid than the other one.

While observing a squealing bee, the saddle-shaped subcosta (fig. 1, *Sc*) rotated quickly on the head of the radius (*R*) which also vibrated; the bases of the cubitus (*Cu*), first and third anal veins (*1A* and *3A*), and the membranes (*Me*) between them likewise vibrated, and the median plate (*MP*) and tegula were observed to move slightly. When the tegula which covers the axillaries was pulled off, the first, second, and third axillaries (*1X*, *2X*, and *3X*) and the membranes (*Me*) between them were seen to vibrate.

It was not possible to observe the ventral surface (fig. 2) of the base of the wing on the living bee, but a study of its anatomy shows that this surface is better adapted to produce sounds than is the dorsal surface (fig. 1). Reference to figure 2 shows that there is twice as much membrane capable of being vibrated on this surface as on the other surface, due to the fact that the subcosta (*Sc*), head of the radius (*R*), and median plate (*MP*) are considerably smaller than they are on the other side. In fact, all the membranes, represented by dots in figure 1, were observed to vibrate, and all of those in figure 2, likewise represented, also probably vibrate. Thus it is evident that the extreme bases on these wings make a good sound-producing organ.

Figure 1 is partly copied from Snodgrass ('10), but the present writer carefully verified all the sclerites here represented, and then made a careful study of the ventral surface (fig. 2), which the former writer did not illustrate. Relative to the muscles, attached to the axillaries, and to the mechanism producing the wing motion, the reader is referred to Snodgrass' bulletin, page 65.

In this study the group of olfactory pores on the front wings have been more carefully observed than they were formerly by the writer ('14 a). Instead of three groups, there are four groups of them; the fourth group, now numbered *1a* in figure 2, was formerly overlooked in superficial observations, but was called no. 2 in figures 19 and 20, page 328. Groups 1, *1a*, and 2 are really located on the head of the radius (fig. 2, *R*), and not on the subcosta, and group 3 lies on the other side of the same sclerite (fig. 1), and not on the median plate.

2. Sound-producing organs of other insects

Pemberton ('11) experimented with several species of Syrphidae, house-fly, honey-bee, and bumble-bee. He says that they do not produce audible sounds by the spiracles or tracheae, but that all humming or buzzing sounds made by them are produced solely by the wings, either by their vibration in the air or by the wing bases striking against the body wall. This author did not study the anatomy of the wing bases.

Aubin ('14), as well as Pemberton, used the syrphid or common drone-fly (*Eristalis tenax*) in all his detailed experiments. The former author, after experimenting with this fly and after carefully identifying all the parts in the bases of its wings, concludes that the buzzing sound is made by a rapid vibration of certain thoracic muscles, attached to a particular sclerite, which strikes the thorax at a given point. The resonant apparatus, consisting of another sclerite and its attached membranes, is thrown into a state of vibration, producing the buzzing sound which is about an octave higher than the humming noise, made by the distal portions of the wings. In the honey-bee, according to the observations of the present writer, no part of the wing base strikes the thorax during the vibration. Aubin believes that, according to the laws of acoustics, the resonant areas in the wing base of this fly might respond to the buzzing of other flies and thus form one of the elements of an auditory apparatus. If this were true, a nervous connection would be necessary. In all probability, no such connection exists in this fly, and certainly not in the honey-bee.

Judging from the known sound-producing apparatus, and so-called auditory organs in crickets, grasshoppers, and katydids, the males are usually neither deaf nor dumb, but the females are always dumb, although not generally deaf. The males of crickets, katydids, and of some grasshoppers make sounds by rubbing their wings together, whereas other grasshoppers make sounds by rubbing the hind legs against the wings. Both sexes possess so-called ears, which in crickets and katydids (*Locustidae*) are found on the front tibiae, but in grasshoppers (*Acrididae*) on the abdomens. As far as known, the female cicada is both

deaf and dumb, but her mate is only deaf, his sonorous sound-producing organ being found in the abdomen. "Happy is the cicada, since its wife has no voice," says Xenarchos, could just as well be said about the males of crickets, grasshoppers, and katydids. Graber, after cutting off the front tibiae of crickets and katydids, found that they responded as well to a violin and to their chirping and singing as before the operation.

Stridulation, special sound-producing apparatus, and various types of supposed auditory organs have been described in true bugs, moths and butterflies, flies and mosquitoes, beetles, and ants, and also in a few larvae and pupae, yet we know very little about this subject.

SO-CALLED AUDITORY ORGANS OF INSECTS

Since insects have special sound-producing organs, it is natural to suppose that they also have auditory organs. The so-called auditory organs of Orthoptera and of certain other insects, mentioned above, need not be further discussed here, because Comstock ('20, pp. 145-154) has recently given a good summary on this subject.

Supposed auditory organs of honey-bee

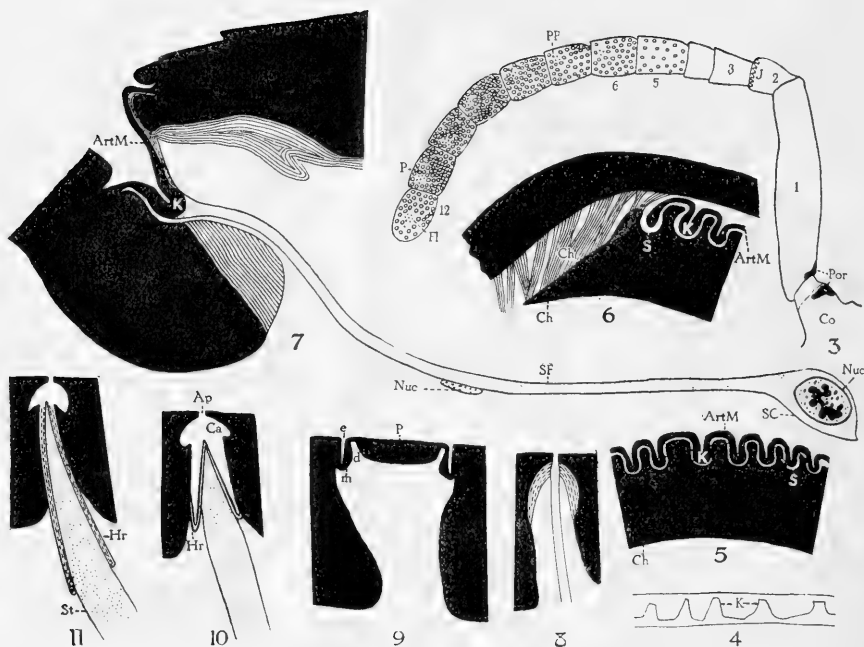
In the following pages the descriptions of five supposed sound receptors are given, and Janet ('11) mentions a sixth one in the bee. From his brief description and drawing the details of this one cannot be interpreted. The following is all that Janet says about it: The chordotonal nerve departs from the antennal nerve a short distance from the brain, and runs toward the integument where it is inserted at a point beneath and a short distance from the articular edge of the antenna. From this point of insertion departs one end of a fusiform chordotonal ganglion, whose other end gives rise to a terminal cord which runs toward the articular membrane of the antenna, and is there inserted. The present writer has not studied this organ, but from the above brief description he would eliminate it as a possible auditory organ.

a. Structure of Johnston's organ. Johnston ('55) pointed out a supposed auditory organ in the second antennal segment of the

culex mosquito. This structure, later called Johnston's organ, was thoroughly investigated by Child ('94 a, b), who saw it in all the insect orders examined, except one. He found it in several genera of Diptera, one genus of Hymenoptera, and in one or more genera each of Coleoptera, Lepidoptera, Neuroptera, Pseudoneuroptera, and Hemiptera (Homoptera). Apparently he did not examine the honey-bee, but found it in a wasp (*Vespa vulgaris*) well developed, although the articular membrane to which the sense cells are attached is not complicated as he found it in mosquitoes and as the present writer saw it in the honey-bee. Of the many specimens examined, Child found this organ most highly developed in the male mosquito (Comstock, '20, pp. 152-154, for a general description). He also saw sense organs in the second antennal segments of Orthoptera, but decided they were not Johnston's organs. Recently these have been described as olfactory pores by the present writer ('20).

The distal end of the second antennal segment (fig. 3, 2) is considerably larger than the proximal end, but the proximal end of the third segment (3) is the narrowest portion of the antenna. When examining the extreme distal end of the second segment under a low-power lens, a circle of irregular structures (*J*), somewhat resembling a miniature mountain chain in shape, passes completely around the segment. Observing a crushed segment under a high-power lens, it will be noted that these structures, known as chitinous knobs (fig. 4, *K*) from now on, lie in the articular membrane between the second and third segments. The top line in figure 4 represents the union of this membrane with the second segment, and the bottom line the union of the same membrane with the third segment. As an average for each caste, a worker has 70 of these knobs; a queen, 72, and a drone, 100.

Oblique sections through the articular membrane show that it (figs. 5 and 6, *ArtM*) is very thin, that the ends of the knobs (*K*) fit into sockets (*S*) in the chitin (*Ch*) of the third segment, and that soft, flexible strands of chitin (figs. 6 and 12, *Ch*) firmly bind the two segments together. In fact, the hard, rigid chitin (represented by solid black) of the articular membrane (figs. 7 and 12, *ArtM*) is reenforced by a layer of soft, flexible



Figs. 3 to 11 Antennal sense organs of honey-bee. Fig. 3, dorsal surface of right antenna of worker, showing following: Two groups of olfactory pores (*Por*) on condyle (*Co*) and scape (*1*); flagellum, consisting of second to twelfth segments (*2* to *12*), bearing Johnston's organ (*J*), pit pegs (*PP*), pore plates (*P*) and Forel flasks (*Fl*); the tactile hairs and pegs are not represented; $\times 20$. Fig. 4, superficial appearance of Johnston's organ on worker antenna, showing knobs (*K*); $\times 320$. Figs 5 and 6, oblique sections through Johnston's organ in drone antenna, showing knobs (*K*) of articular membrane (*ArtM*) in sockets (*S*) of chitin (*Ch*), and soft, flexible strands of chitin (*Ch*) which firmly bind second and third segments together; $\times 500$. Fig. 7, from three consecutive longitudinal sections of nineteen-day-old worker antenna, showing sensory part of Johnston's organ, consisting of sense cell (*SC*), its nucleus (*Nuc*) and sense fiber (*SF*) and probably the latter's nucleus (*Nuc*); note distal end of sense fiber attached to knob (*K*) of articular membrane (*ArtM*); $\times 1000$. Figs. 8 to 11, internal structure of antennal organs; $\times 1000$. Fig. 8, olfactory pore from worker condyle. Fig. 9, pore plate from drone antenna, showing plate (*P*), two grooves (*d* and *e*), and double hinge-like membrane (*m*). Figs. 10 and 11, pit peg and Forel flask, respectively, from worker antenna, showing semitransparent hair (*Hr*), nerve strand (*St*), cavity (*Ca*), and aperture (*Ap*).

chitin (represented by broken lines). Consequently, instead of this articulation being weak, it is as strong as any other, and when broken by a steady pull, the articular membrane remains fastened to the third segment, showing that the knobs, although having considerable play in their sockets, nevertheless lend considerable strength to the articulation.

Longitudinal sections through the second antennal segment show the following: A large group of sense cells (fig. 12, *SC*) lies on either side of the section; two large antennal nerves (*N*), called internal and external olfactory nerves by Janet ('11), run through the center of the segment and at various places unite with the groups of sense cells, as shown in figure 12, and a large trachea (*Tr*) runs near the nerves and sends out branches here and there.

A thorough study of these sections under an oil-immersion lens shows the following: The elliptical sense cells (fig. 7, *SC*) have conspicuous nuclei (*Nuc*), short nerve fibers (fig. 12, *NF*) which run into the nerves, and long and comparatively large sense fibers (*SF*) which run in bunches toward the articular membrane. About half-way between the sense cell and articular membrane may be seen small slender nuclei (fig. 7, *Nuc*), some of which seem to lie on the surface of the sense fibers, but it is more likely that these are hypodermal nuclei, although the nuclei in the hypodermis (fig. 12, *Hyp*) usually are round and much larger. When the bunches of sense fibers reach the flexible strands of chitin (*Ch*) the individual fibers separate, run between these strands, then unite singly with the inner ends of the knobs (*K*).

Figure 12 is a diagram showing most of the second segment in longitudinal section and in perspective, and a small portion of the third segment in both cross and longitudinal section and in perspective. It is noted that the thin articular membrane (*ArtM*), bearing the chitinous knobs (*K*), is unprotected and fully exposed to the outside air. Two of the knobs are cut lengthwise, showing the cone-shaped cavity which opens to the exterior. The other knobs are heavily shaded, showing that they are buried in the articular membrane.

A glance at figure 12 shows that the articular membrane resembles the head of a drum and that the knobs act chiefly as sense-fiber attachments. It is evident, judging merely from the structure of this organ, that gusts of wind and possibly weak air currents would cause the articular membrane to vibrate, thereby irritating the sense cells. This organ might also receive jar

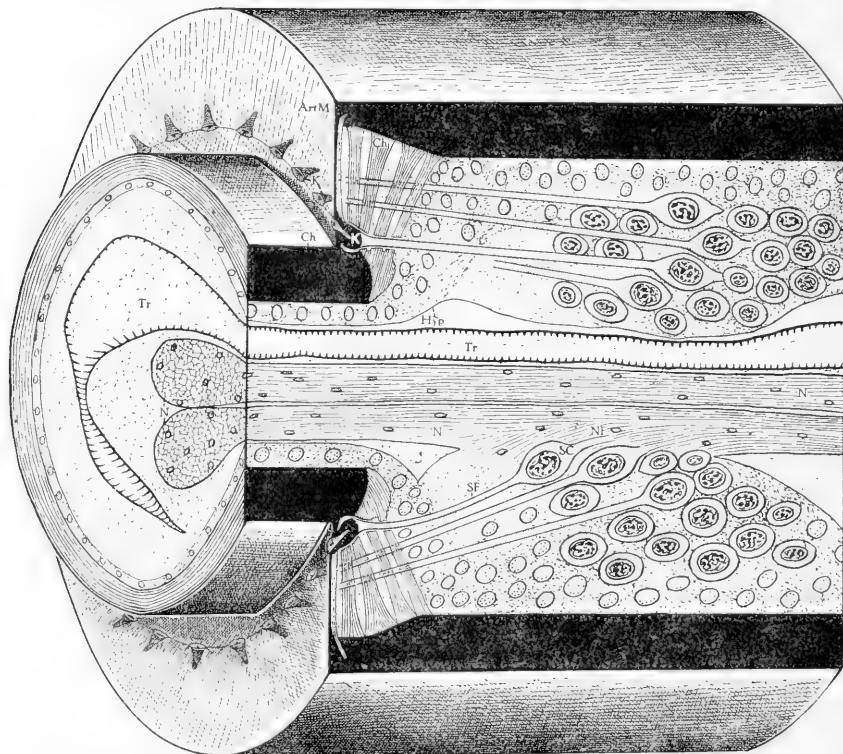


Fig. 12 Diagram, representing most of second antennal segment of worker honey-bee in longitudinal section and in perspective and a small portion of third segment in both cross and longitudinal section and in perspective, showing Johnston's organ which consists of two large groups of sense cells (*SC*) whose nerve fibers (*NF*) run into the two antennal nerves (*N*) and whose sense fibers (*SF*) are attached to the knobs (*K*) in the articular membrane (*ArtM*). Two of the knobs are cut lengthwise, showing the cone-shaped cavity which opens to the exterior, while the other knobs are heavily shaded, indicating that they are buried in the articular membrane. *Tr*, trachea; *Hyp*, hypodermis; *Ch*, hard chitin; and *Ch*₁, soft, flexible strands of chitin which firmly bind second and third segments together.

stimuli, but it appears too crude to act as a sound-wave receptor, unless it is able to receive sound vibrations of a very low frequency. The most reasonable function that the writer can think of is the one suggested by Demoll ('17), that it may serve as a statical organ to register the movements of the flagellum. Since there can be no muscular sense in the flagellum, because this part of the antenna possesses no muscles, such an organ would seem very useful. The scape or first antennal segment (fig. 3, 1) contains many muscle fibers, most of which run to the articulation between the first and second segments. These muscles can only move the flagellum about in all directions, but cannot bend it. Since the antenna is the chief tactile organ of the bee and must be carefully operated, the only way of bending the many-jointed flagellum is by blood pressure. The blood bathes all the internal structures, and consequently any change in its pressure would affect the articular membrane. Even if the Johnston's organ in the honey-bee receives sound vibrations of a low frequency, or functions in any other way suggested above, we should probably classify it as a tactile organ rather than as an auditory organ.

Child ('94 b) says that the function of Johnston's organ is in general to receive original touch stimuli; it can, however, in a broader sense receive the stimuli of sound vibrations. The auditory stimuli are to be thought of as modified touch stimuli. When the same organ serves both as touch and auditory receptors, as is possible in mosquitoes and midges, then the insect will be able to differentiate between the touch response and auditory response.

According to Child, this organ is of hypodermal origin, arising from a ringlike fold near the antennal funnels which are invaginated in the head.

Several years ago the writer discovered two groups of olfactory pores on the base of the antenna, but they are here described for the first time. One group of about twenty-five pores lies among a bunch of tactile hairs on the distal end of the articular knob or condyle (fig. 3, *Co*), and the other group (*Por*) of twelve pores lies on the proximal end of the scape. So far as known, these are

the only olfactory pores on the antenna of the honey-bee. The external and internal structure (fig. 8) are like those already described by the writer ('14 a).

b. Structure of pore plates. The pore plates or sensilla placodea, according to the writer's discussion of the antennal organs ('14 b), were first studied in 1847 by Erichson, who called them olfactory organs. Since this date they have been studied by about three dozen other investigators whose views concerning their function differ widely. In 1851 Vogt suggested that they perform a function combining those of smell and touch. In 1858 Lespés compared them to the ears of higher animals, and a year later Hicks called them auditory organs. Practically all of the other authors up to 1888, who have studied the pore plates, regard them as olfactory organs. Ruland ('88), after having boiled antennae in caustic potash, saw that a pore plate is suspended on a membrane, resembling a double hinge, similar to that observed in sections stained in eosin by the present writer. Owing to this arrangement, he called them auditory organs. In 1894 Nagel favored the olfactory view, but also thought that the pore plates might have a mechanical function. He suggested that air pressure might affect them. Nine years later Schenk ('03) stated that the thick plates in these organs eliminated the possibility of these structures being olfactory organs, but judging from their anatomy he regarded them as having a mechanical function. He favors the view that they are pressure points to inform the bee of the object immediately in front of it.

According to Schenk's calculations, a male honey-bee has 31,356 pore plates and a female has only 3,648. According to the calculations of the present writer, a drone on an average has 29,718 pore plates; a worker has 4,744, and a queen has 2,776. Those of the drone are much smaller than those of the worker or queen, but supposing that their sensitiveness is in direct proportion to the total area of all their plates, then if the sensitiveness of those on a worker equals 1, that of those on a drone equals 3, and that of those on a queen equals only 0.6. These organs (fig. 3, *P*) are found only on the fifth to twelfth antennal segments of the worker and queen, and on the fifth to thirteenth segments

of the drone. They are rather equally distributed over the various segments. Using the average number of pore plates on a worker antenna as an example, the segments and number of these organs are: 5th, 322; 6th, 345; 7th, 332; 8th, 288; 9th, 284; 10th, 283; 11th, 278, and 12th, 240. Twelve per cent of these lie on the ventral surface and 88 per cent on the dorsal surface. Relative to the pore plates on the queen and drone, only 3 per cent of those of the former and 25 per cent of the latter lie on the ventral surface of the antenna.

Viewed superficially with transmitted light, a pore plate (fig. 13, *P*) is seen to consist of an elliptical light spot, which is surrounded by three concentric bands; the first and third ones (*a* and *c*) being light in color, and the second or middle one (*b*) being dark. A section through this organ shows that the hard and thick plate (figs. 9 and 13, *P*) is suspended on a membrane (*m*), resembling a double hinge, which viewed by transmitted light causes the above dark band (*b*), while an inner groove (*d*) causes the first light band (*a*) and an outer groove (*e*) produces the other light band (*c*). In reality this outer groove is not a true groove, because its walls or sides lie against each other and allow no cavity, except perhaps when the plate is vibrated. This fact explains why other observers have overlooked it. Ruland saw it in sections made from caustic potash material, which must have been considerably distorted. The present writer has also seen it many times in the same kind of sections, besides in other sections made from material not treated with KOH. Any dark stain obliterates this groove, and consequently the writer was able to see it by using eosin alone.

Judging from the structure of a pore plate, the elliptical plate (fig. 13, *P*) may be moved in and out on the double hinge (*m*), thereby moving the large nerve strand (*St*) and consequently affecting the large sense cell group (*SCG*). These organs, therefore, might be an air-pressure apparatus, as suggested by Nagel and Schenk. It has been observed by Schenk and the present writer that bees, when flying toward an object, such as a window, light on their feet instead of butting their heads into the object. Now, it may be that the pore plates act as an air-pressure appara-

tus, in which capacity they inform the insects of the objects immediately in front of them. In case of the honey-bee, they might also be sensitive to the weak currents of air caused by workers fanning. It is possible that the sense hairs are not affected by these weak currents, and therefore some method is

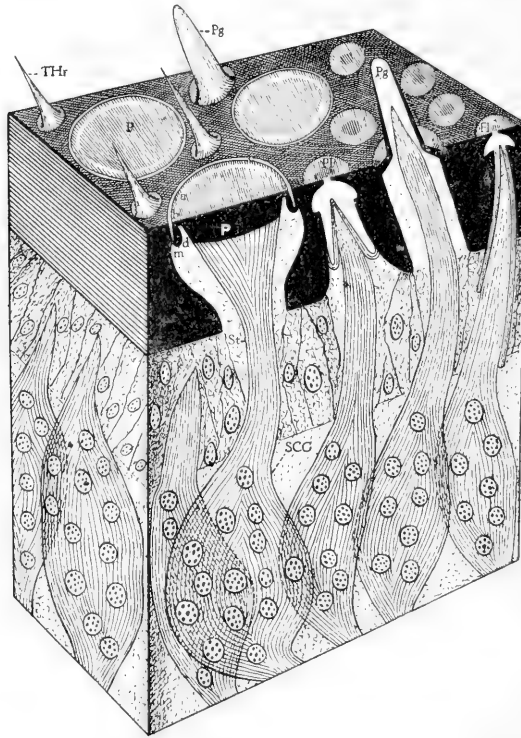


Fig. 13 Diagram, representing a block taken from terminal antennal segment of worker honey-bee, showing tactile hairs (*Thr*), pegs (*Pg*), pit pegs (*PP*), Forel flasks (*Fl*), and pore plates (*P*) in both perspective and in section. *a* and *c*, light colored bands; *b*, dark band; *d*, inner groove; and *m*, double, hinge-like membrane of pore plate; *St*, nerve strand; *SCG*, sense cell group.

badly needed to keep the bees constantly informed whether or not the fanners are working properly. If these interpretations are correct, we here have another form of touch. Of course these organs might also be sensitive to wave vibrations of a very low frequency, but if this interpretation is correct, we would yet

probably be more correct by classifying them as tactile organs rather than as auditory perceptors.

c. Structure of other antennal organs. Relative to the other antennal organs, there are four, all of which are really hairs. The tactile hairs (fig. 13, *THr*) or sensilla trichodea are scarce on the antennae of the male honey-bees, but numerous on the antennae of the females. They are regarded by all the observers as tactile organs. The pegs (*Pg*) or sensilla basiconica are absent in the males, but numerous on the antennae of the females. They are generally considered as olfactory organs, because their tips are covered with very thin chitin. The present writer believes that they are very delicate touch organs.

The pit pegs (fig. 13, *PP*) or sensilla coeloconica and Forel flasks (*Fl*) or sensilla ampullacea are hairs inside of pits. On the antennae of the males both of these types are somewhat numerous, but on the antennae of the females they are comparatively scarce. Viewed superficially, one type cannot be distinguished from the other, but sections show that the pit pegs are usually the larger in diameter. Relative to the antennae of workers, most of these organs (fig. 3, *PP* and *Fl*) lie in groups on the sixth to twelfth segments, and counting both types combined there are not more than 100 individual organs on each antenna. In regard to their internal structure, they differ somewhat, as may be seen by referring to figures 10, 11, and 13. In both types the semitransparent hair (*HR*) ends in a cavity (*Ca*), which communicates with the exterior by a minute aperture (*Ap*), and each hair is connected with a nerve strand (*St*), which runs to a sense cell group (*SCG*). The function of these organs is usually regarded as problematical, but still a few authors have called them auditory organs. The present writer has no conception of what their function is, but for some time he has looked upon them as more or less degenerated structures.

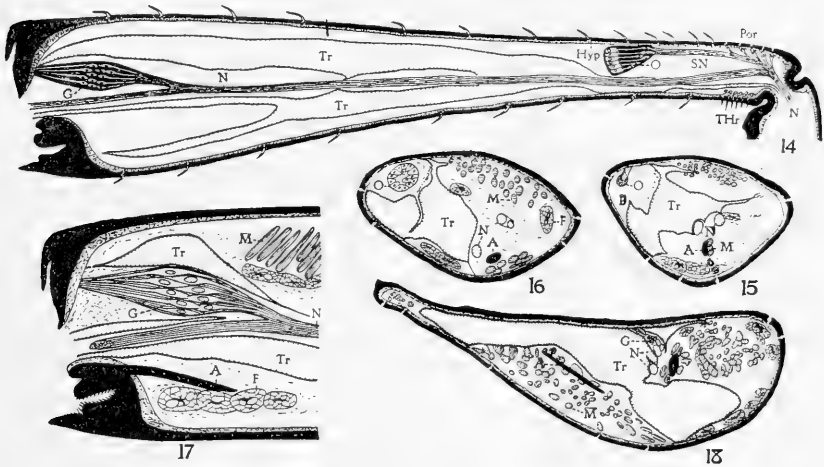
At this place the writer wishes to call attention to an erroneous idea which text-book writers still persist in handing down. Before understanding the internal anatomy of antennae, some of the early microscopists imagined that they saw gland cells among the masses of sense-cell groups. This led to the idea that in order

for the antennal organs to function as olfactory or gustatory organs the secretion from these glands must pass through the thin chitin and must keep the outer surfaces of the organs moist and thus fitted for the reception of chemical stimuli. This is nice in theory, but there is not one iota of truth in such an assumption, because not one of the later investigators mentions having seen glands connected in any way with the antennal organs. Ruland in 1888 denies the presence of them, and the present writer has never seen anything in the antennae which he could call glands. Berlese ('09, p. 610) maintains that the essential feature of these chemical sense organs is the presence of antennal glands, and Comstock ('20, p. 133) quotes Berlese on this subject and then describes various types of hairs which have been called organs of smell and taste. The present writer ('16) does not believe that insects have a true gustatory sense and regards it absurd to consider any form of hair capable of receiving chemical stimuli.

d. Structure of tibial chordotonal organs. Schön ('11) described and illustrated the structure and development of the tibial chordotonal organs in the honey-bee and ants. The present writer has carefully studied the structure of the same organs in the honey-bee and differs with Schön only in a few details.

Sections through the tibiae of all three pairs of legs of workers and drones were made and a chordotonal organ was invariably found in each tibia sectioned. It lies (fig. 14, *O*) in the proximal end of the tibia, about one-fourth the distance from the femoro-tibial articulation to the tarsotibial articulation. This portion of the tibia is divided into two distinct chambers by the large trachea (figs. 15 and 16, *Tr*). The blood chamber (fig. 15, *B*) contains only blood and the chordotonal organ (*O*), while the other chamber contains blood, muscles (*M*), apodemes (*A*), nerves (*N*), fat-cells (*F*), etc. In longitudinal sections this organ expands fan-like across the blood chamber and usually appears to be attached by its proximal end to the hypodermis (figs. 14 and 19, *Hyp*), but in other sections where the tibia is considerably compressed both its proximal and distal ends are attached to the hypodermis on the anterior side of the leg (fig. 19). In a series

of cross-sections it may be seen to arise merely as a nerve attached to the hypodermis; then the nerve suddenly runs to a few large cells (fig. 15); a few sections further on the nerve disappears, and the organ assumes a spherical shape (fig. 16, *O*), and the walls are lined with a thick layer of large cells, leaving a cavity in the center, which is apparently filled with a liquid (probably only blood).



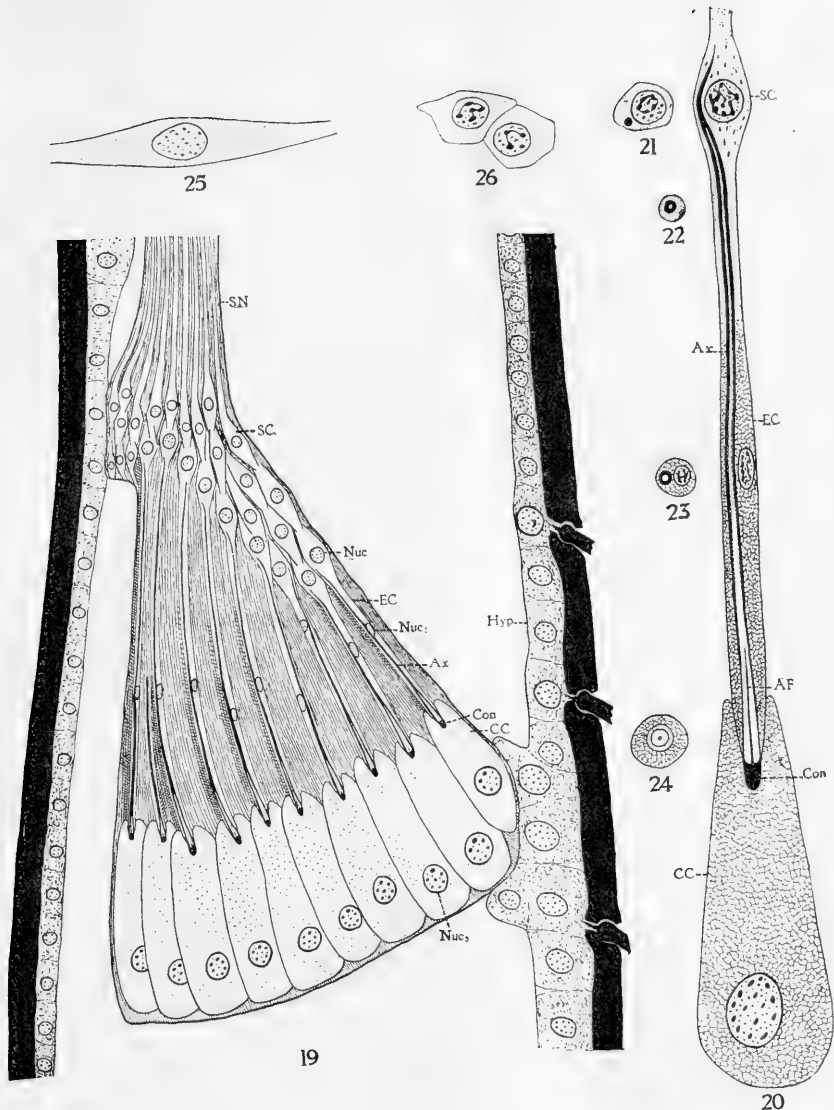
Figs. 14 to 18 Sections, showing structure of tibial sense organs. Figs. 14 and 17, each a semidiagrammatic drawing from two consecutive longitudinal sections of hind tibia of a drone, showing relative position of tactile hairs (*THr*), olfactory pores (*Por*), chordotonal organ (*O*), and ganglion cells (*G*), all of which are innervated by the same nerve (*N*, too wide here), although a small branch of this nerve, called the subgenual nerve (*SN*) runs directly to the chordotonal organ; fig. 14, $\times 32$, and fig. 17, $\times 53$. Figs. 15, 16, and 18, cross-sections through tibia of a worker, showing relative position and shape of chordotonal organ (*O*) and ganglion cells (*G*); $\times 53$; fig. 15, through proximal end of group of ganglion cells. *A*, apodeme; *B*, blood chamber; *F*, fat cell; *Hyp*, hypodermis; *M*, muscle; *N*, two main branches of nerve; *Tr*, trachea.

Using Schön's terminology, the detailed description of a single chordotonal organ is as follows. The tibial nerve after immediately emerging from the femur is apparently divided into two large branches, because in all of the cross-sections made two comparatively large nerves (figs. 15, 16, and 18, *N*) were found. A short distance from the femorotibial articulation one

of the branches gives off fibers to some tactile hairs (fig. 14, *THr*) and also a smaller branch which innervates the olfactory pores (*Por*) and the chordotonal organ (*O*). Schön calls this small branch (figs. 14 and 19, *SN*) the subgenual nerve (Subgenualnerv). It runs into the sense cell group and gives off a fiber to each individual sense cell (fig. 19, *SC*). The spindle- but sometimes diamond-shaped sense cells lie in a mass which extends diagonally half-way across the blood chamber. In all of the present writer's sections, the organ is anchored at the base of the sense cell group, but only occasionally was it also fastened at the other end of the organ. According to Schön, it arises from the distal end and should always be fastened at this end. The distal end of a sense cell is terminated into a long, slender, sac-like enveloping cell (*EC*, Umhüllungszelle) whose elongated nucleus (*Nuc*₂) sometimes nearly fills the entire lumen of the cell. Running the full length of the enveloping cell there is a dark-staining thread, the axial tube (*Ax*), which ends in a much darker staining body, the cone (*Con*, *Stift*), lying in the proximal end of the large oblong or pear-shaped cap cell (*CC*, Kappenzelle) whose nucleus (*Nuc*₃) usually lies in the distal end.

The walls of the axial tube correspond to the extended walls of Schön's *Stift*. Schön also describes two other types of cells which the present writer has not been able to differentiate from those just mentioned. His accessory cells (akzessorische Zellen) lie between the cap cells and his end fibers (Endfasern), which fasten the organ to the hypodermis.

A more careful study of the sensory element of the chordotonal organ under a magnification of 1900 diameters shows the following: Lying around the conspicuous nucleus (figs. 19 and 20, *Nuc*) of the sense cells (*SC*) there are large dark-staining particles, the largest one of which seems to be the tail end of the axial tube (*Ax*). In longitudinal sections this particle appears as a dark streak and may or may not reach as far as the nucleus. In cross-sections it appears as a large, dark, solid particle (fig. 21). Cross-sections through the proximal end (fig. 22), middle portion (fig. 23), and distal end (fig. 24) of the enveloping cell (*EC*) show that instead of the axial tube continuing very far as a rod,



Figs. 19 to 26 Sections, showing detailed structure of chordotonal organ and ganglion cells. Fig. 19, semidiagrammatic drawing from several longitudinal sections through anterior portion at proximal end of hind tibia of a drone, twenty-one days old, showing chordotonal organ suspended in blood chamber, but attached at both ends to hypodermis (*Hyp*); $\times 320$. Fig. 20, longitudinal view of one of the sensory elements from fig. 19, showing following parts in detail; sense cell (*SC*), axial tube (*Ax*), enveloping cell (*EC*), axial fiber (*AF*), cone (*Con*), cap cell (*CC*), and nuclei (*Nuc*₁, *Nuc*₂ and *Nuc*₃) of sense cell, enveloping cell and cap cell, respectively. Fig. 21, cross-section of sense cell (*SC*) through nucleus, showing tail end (large dot) of axial tube; figs. 22, 23, and 24, cross-sections of proximal end, of middle portion through nucleus, and of distal end, respectively, of enveloping cell, showing appearance of axial tube (*Ax*) and its fiber (*AF*). Fig. 25, longitudinal section, and fig. 26, cross-section of ganglion cells from tibia of a drone. Figs. 20 to 26, $\times 1000$.

it becomes a tube with thick walls at first, but the walls gradually become thinner and thinner as one glances at them from the proximal end to the distal end. The outer layer of the walls seems solid, often does not take the stain, but remains a light yellow color. The inner layer does not appear totally solid, but usually is stained more or less. The axial tube seems harder than the surrounding tissue and occasionally the microtome knife fails to cut it, thus leaving it slightly projecting from a cross-section. Hence from all appearances the axial tube is a semichitinous structure.

The head end of the axial tube terminates in the cone (fig. 20, *Con*), from the center of which arises the short axial fiber (*AF*), scarcely visible under the highest magnification. In position this fiber corresponds to the Schön's axial fiber, which extends the full length of the enveloping cell. Figure 24 shows the relation of the various parts in cross-section just in front of the cone. The dot represents the axial fiber; the inner circle, the walls of the axial tube; the middle circle, the walls of the enveloping cell, and the outer circle, the walls of the cap cell.

The cytoplasm in the distal half of the enveloping cell and in the cap cell (fig. 20, *CC*) appears slightly net-like, although this appearance is exaggerated in figures 20, 23, and 24.

In regard to the development of the chordotonal organ, Schön says that eight days after the honey-bee egg is laid a small growth projecting into the blood chamber is seen developing in the hypodermal cells in the tibia from which the organ arises. On the ninth day may be seen the first differentiation of cells, and on the tenth and eleventh days one may distinctly see sense cells, enveloping cells, and cap cells. On the eleventh and twelfth days the cone is formed, and on the thirteenth day the nervous part of the organ is laid down in the blood chamber. On the fifteenth and sixteenth days the end fibers are developed, and on the seventeenth day the organ is fully developed. It is of a purely ectodermal formation.

Schön says that since the trachea is so greatly expanded where the chordotonal organ occurs, it may probably have something to do with the function of this organ; but the present writer does

not think so, because the trachea does not come in contact with the organ and is no more expanded here than in other places in the tibia.

So far no external device or apparatus connecting with the internal organ has been found, although Schön imagined that he had found the external portion when he thought he saw two rows of sense cones (Sinneskegel) on the proximal end of each tibia. In position and number these cones correspond exactly to the olfactory pores described by the present writer. When observed without the cylindrical tibia being properly rotated, they often externally resemble cones; but when the tibia is rotated slightly, so that they lie on the median line of the tibia, the optical illusion becomes evident. Schön found that both the chordotonal organ and these imaginary cones are innervated by the subgenual nerve, and consequently he believes that the cones act as the external apparatus of the organ. Schön describes and illustrates the internal anatomy of his sense cones (the present writer's olfactory pores), but here does not recognize them as cones, for he follows vom Rath by calling them membrane canals (Membrankanäle).

Nothing can be said about the probable function of the chordotonal organ, but if it were connected with an external apparatus, similar to that found in some Orthoptera, it might serve as an auditory organ.

Schön says that there is a great similarity between this organ in Hymenoptera and that in Orthoptera. These organs in bumble-bees, wasps, and Terebrantidae vary greatly with those in ants and honey-bees. In all the organ is fastened to the hypodermis and in all he found the sense cones and spindle-shaped sense cells which with the subgenual nerve protrude into the nervous ends of the enveloping cells.

e. Structure of tibial ganglion cells. In the first longitudinal sections made, the writer observed a group of supposedly sense cells which he thought was associated with the chordotonal organ, but after studying more sections it was ascertained that these cells are totally independent of the chordotonal organ, because the former (fig. 14, *G*) are located at the distal end of the tibia

and the latter (*O*) at the proximal end of the tibia, and no connection could be found between them, except that the same nerve (*N*) sends off a branch to each group of cells.

For lack of a more appropriate name, the cells under discussion may be called tibial ganglion cells, although the writer knows of no similar group in insects. Schön apparently did not see them and perhaps this is the first time for them to be described. They lie in a mass (fig. 17, *G*) between two tracheae (*Tr*) at the extreme distal end of the tibia. The distal end of the group is attached to the hypodermis near the articulation, while the proximal end terminates in a branch of the main nerve.

Figure 18 is a cross-section showing this group of cells (*G*) just departing from the nerve (*N*), and some of the fibers may be seen between the two tracheae (*Tr*) running to the hypodermis.

This group of cells is slightly larger than the chordotonal organ, but the individual cells (figs. 25 and 26) in it are practically the same in shape and size as are the sense cells in the chordotonal organ.

SUMMARY

Bee-keepers are agreed that bees can hear, yet they cannot prove it, and critics still contend that it has never been experimentally proved that any insect can hear; nevertheless, within the last few years some good experimental results have been obtained.

The special sound-producing apparatus of the honey-bee consists of the membranes lying between the axillaries at the bases of the front wings. Muscles, lying in the thorax and attached to these axillaries, contract and relax very quickly, thereby causing the axillaries to vibrate; consequently, the above membranes are caused to vibrate rapidly, thus producing the piping, teeting, or squealing noise commonly heard when a bee is squeezed.

Up to date five so-called auditory organs have been found in the honey-bee. Judging from their anatomy, the pore plates, Forel flasks, pit pegs, and Johnston's organ, all located in the antennae, do not seem to be well fitted to act as sound receptors; but the chordotonal organs, lying in the tibiae might be better

adapted for this purpose, providing they had an external portion, corresponding to the tympanum.

The Johnston's organ, lying in the second antennal segment, consists of the peculiarly modified articular membrane between the second and third antennal segments and of many sense cells whose fibers unite with peculiar knobs extending inwardly from the articular membrane. This organ does not seem well adapted to act as an auditory organ unless it is able to receive sound vibrations of a very low frequency. It might also be sensitive to weak air currents and possibly to jars, but the most reasonable function that the writer can think of is that it may serve as a statical organ to register the movements of the flagellum.

The pore plates, lying so abundantly on the antennae and called olfactory organs by most of the other authors, were found to have two grooves encircling each elliptical plate, thereby allowing the plate to move in and out on a double hinge. Judging from this mechanism, the pore plates might act as an air-pressure apparatus to inform the bees of an object immediately in front of them, and thus prevent them from striking against objects. They might also be sensitive to the weak air currents made by workers fanning, thereby serving as an apparatus to keep the bees constantly informed whether or not the fanners are working properly.

The functions of the Forel flasks and pit pegs are problematical.

The chordotonal organs, found in the proximal ends of the tibiae, are very complicated in structure and are similar to those found in the tibiae of crickets and katydids, but the former do not have external membranes, while the latter do. Nothing can be said about the function of the chordotonal organs in honey-bees.

A group of ganglion cells was found in the extreme distal end of each tibia, but nothing can be said about its function.

In conclusion, it may be that the sense of hearing in insects is on no higher plane than that advocated by Forel ('08), who believes that insects do not hear, at least as we do, but compares this perception in them to that in deaf-mutes who feel the rolling of a carriage at a distance. Forel says:

Hearing is a physical sense. Sonorous waves, especially those of low sounds, are nearer to large mechanical vibrations than luminous, caloric, or electric waves. Hearing, therefore, must be in its origin connected with touch, but we make a distinct difference between the perception of a very low sound by touch and its perception by hearing. We must not forget that the specialization of the organ of hearing has reached in man a delicacy of detail which is evidently not found again in lower vertebrates. It is, I believe, the sense which removes us most from the lower animals. In animals as high as fish the auditory nerve is confused with other nerves, and the portion of the labyrinth most specially affected by our audition, the cochlea, has disappeared.

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Resumen por el autor, G. W. Bartelmez.

El origen de los primordios ótico y óptico en el hombre.

El presente trabajo se basa en datos obtenidos en doce embriones humanos normales en los estados de cuatro a dieciseis somitas. En el hombre, a diferencia de lo que sucede en la mayor parte de los vertebrados, la placa ótica y su ganglio asociado aparecen muy temprano, y hasta preceden al primordio óptico. La placa ótica aparece enfrente de la segunda división del cerebro posterior, y el ganglio cerca del borde dorsal del pliegue neural adyacente. En los estados de nueve y diez somitas las células del esbozo gangliónico comienzan a perder su disposición epitelial y la masa entera se separa del pliegue neural. Se deriva de la pared del futuro tubo neural. Durante este periodo el epitelio ótico se diferencia de una manera característica y después se invagina.

El esbozo óptico puede reconocerse por vez primera como un engrosamiento de los pliegues neurales, correspondiente a la región del cerebro anterior. En este primordio de la "cresta óptica" se produce lateralmente la vesícula óptica; de las porciones media y caudal parte una proliferación de la cresta neural. El surco óptico puede reconocerse en el estado de ocho somitas. La evaginación se dirige al principio ventralmente, pero al aproximarse los pliegues neurales se dirige lateralmente hacia el ectodermo. En el estado de dieciseis somitas la vesícula óptica está en contacto con el ectodermo. Se deriva enteramente de las paredes del tubo neural definitivo, porque una parte de la pared del futuro cerebro dorso-lateral interviene desde el principio entre el esbozo óptico y el ectodermo.

THE ORIGIN OF THE OTIC AND OPTIC PRIMORDIA IN MAN

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TEN FIGURES

INTRODUCTION

The early history of the nervous system in man and in the great majority of other mammals is but imperfectly known. In the whole order there is only a single detailed study of the origin of the cranial ganglia. The data on this phase of human development are for the most part included in descriptions of single specimens; they are few and incomplete and marred by faulty interpretations due to the lack of a human series and neglect of comparative material from other forms.

We shall here confine our attention to the otic and optic primordia, although the identification of them in the various embryos of the series is based in part upon our interpretation of the primary subdivisions of the nervous system. This evidence will be presented in a subsequent paper.

MATERIAL

The present observations are based upon the study of complete serial sections of twelve normal embryos ranging between stages of three and sixteen somites. In addition, the data from the published descriptions of four others have been available, so that the series is a reasonably complete one, even though some of the specimens leave much to be desired in the matter of histological detail. We have models of eight of the embryos, and in the case of some of them several models were prepared. The specimens studied in this connection are as follows:

	DESIGNATION OF EMBRYO	NUMBER OF SOMITES	LENGTH	CONDITION
1	H279 Univ. of Chicago Coll.	4	2.5 mm. in formol	Fair, abundant mitoses
2	'Klb.' Normentafel no. 3	5 to 6	1.8 mm. in alcohol	Excellent
3	# 391 Carnegie Coll. (Dandy 1910)	8	2 mm. in formol	Fair
4	H87 Univ. of Chicago Coll.	8	Circ. 2 mm. in formol	Good
5	Eternod embryo 'DuGa' (1896)	Probably 9	2.12 mm. from number of sections in the series	Excellent
6	H637 Univ. of Chicago Coll.	11	Distorted. 1.85 mm. from number of sections	Excellent histologically
7	H 197 Univ. of California Coll.	12	1.15 mm. (much flexed)	Good
8	H392 Univ. of Chicago Coll.	11	3.6 mm. in formol	Fair clumped mitoses
9	#4 New York Univ. Coll. (Wallin 1913)	14	2.3 mm. after fixation	Good histologically
10	H8 Univ. of Chicago Coll.	14	3.3 mm. in alcohol	Poor
11	'Pfst. III' Normentafel #6	14	Circ. 2.6 mm.	Excellent
12	#470 Carnegie Coll.	Probably 16	3.3 mm. in 95% alcohol	Fair clumped mitoses

ACKNOWLEDGMENTS

This paper is part of a study of human development during the early part of the period of somite formation, begun in 1915 in the Carnegie Laboratory at Baltimore in conjunction with Dr. H. M. Evans (1917). The complete account is to appear

as a joint paper in the Carnegie Institution's "Contributions to Embryology." Although Dr. Evans' own material, his extensive studies on embryos in the European collections and in the Mall collection have been used freely, this part appears under my name as I am assuming complete responsibility for the interpretation of the nervous system. Needless to say, I am under great and varied obligations to Doctor Evans which I gladly acknowledge. The work was begun at the suggestion of the late Dr. F. P. Mall, to whom we owe much. Dr. G. L. Streeter has continued to support it and has helped and advised. Most of the drawings are the work of Mr. J. F. Didusch, whose understanding help has been invaluable.

We would express our appreciation of the courtesy of Profs. Franz Keibel, A. C. F. Eternod, and H. D. Senior for permission to study the young human embryos in their collections.

To the following physicians we are indebted for the embryos of our own series, for their cooperation and for the care which they took to preserve these delicate specimens: Dr. J. P. Spooner, of Peru, Indiana, for H279; Alpheus B. Streedain, of Chicago, for H87; Dr. Edwin Hirsch, of Chicago, for H637; Dr. Robert T. Legge, of Berkeley, California, for H 197; Dr. Ethel Rice, of Chicago, for H392; Dr. J. F. Burkholder, of Chicago, for H8. The exceptional success we have had in obtaining young embryos has been due in large measure to the support and cooperation of Dr. R. R. Bensley.

THE OTIC PRIMORDIUM

The earliest sensory primordium that can be recognized in man is a thickening of the ectoderm opposite the neural folds of the hindbrain. This is the beginning of the otic plate. Several statements in the literature indicate that the otic plate appears early in human development. In 1908 Keibel identified 'die Hörplatte' in the Unger embryo (Normentafel 4), which had about nine somites. Wilson ('14, p. 325) suggested that a pair of diffuse thickenings in his two- to three-somite embryo (H3) might be the 'auditory areas.' Tracing the otic plate back through our series leaves little doubt but that he was correct

in this. Ingalls ('20, p. 67) likewise identified it in his slightly older specimen (Carnegie Collection no. 1878), and with greater certainty, as he had several of our models of older stages for comparison. Figure 1 shows the conditions in the first of our present series, a four-somite embryo (H279) with wide open neural folds. The dorsal part of the fold near the beginning of the second subdivision of the hindbrain (cf. p. 207) is enlarged; a swelling protrudes toward the ectoderm, *ac. fac. gang.*; and there is a



Fig. 1 A photomicrograph of section 54 of the four somite embryo H27 (Univ. of Chicago Coll.). $\times 100$. The plane of section is almost horizontal to the hindbrain. *ac. fac. gang.*, anlage of the acousticofacial ganglion. The overlying cap of ectodermal cells is more deeply stained than the ganglion. *ot. pl.*, otic plate; *am.*, amnion; *y. s.*, yolk sac.

corresponding ventricular sulcus which does not show clearly in the photomicrograph. This is very like the condition described by Schulte and Tilney ('15) in the cat. The enlargement is termed acousticofacial ganglion in accordance with the customary mammalian usage (cf. p. 214). The ganglionic anlage is capped by a single layer of ectoderm cells which appears as if it had slipped over the top of the neural fold. The adjacent ectoderm is obviously thickened as the otic plate, *ot. pl.* The section, which is nearly horizontal through this region, shows almost the

whole rostrocaudal extent of the otic plate. In most sections it fades off gradually into the surrounding ectoderm so that its limits are difficult to determine.

Both the ganglion and the otic plate are present in the six-somite 'Klb.' of Keibel, but our tracings are not sufficiently detailed to permit of an accurate description. At the time they were made the presence of the anlagen was not suspected. They can be found in the eight-somite embryo first described by Dandy ('10) (Mall, No. 391). Figure 2a is taken from a section through the middle of the otic plate of this embryo and shows the ganglionic primordium clearly on the right side. This appears as an outpouching of the neural fold with the characteristic cap of overlying ectoderm.

The 'otic sulcus' is manifest in a model of this region made at 400 diameters. It is a broad shallow pit near the dorsal edge of the fold extending through four of the 10 μ sections. In this case also it belongs to the second hindbrain segment and lies just caudal to the first visceral pouch.¹

The depression to the left in the figure is not the beginning of the otic pit, but a chance wrinkling of the ectoderm.

With the illustrations at hand it will be easier to visualize these relations in the other eight-somite embryo, H87. Figure 3 represents the dorsal aspect of a model and figure 4 is from a projection reconstruction of the embryo cut in the mid-sagittal plane and viewed from the right. The sensory anlagen are plotted in from a detailed study of the sections and indicated by stippling. In the former figure we see the broad expanse of forebrain, still in the neural-plate stage. The deep neural groove has progressed as far forward as the midbrain, behind which are two large hindbrain subdivisions. From the second of these the acousticofacial ganglion is arising and laterally in the ectoderm is the otic plate reaching back as far as the third hindbrain segment. The primary brain segments are better shown in figure 4.

¹ Manifestly our interpretation of the nervous system of this embryo does not agree with that of Dandy ('10). Having identified the cranial flexure, the otic plate and its associated ganglion throughout our series, it has been possible to interpret correctly the subdivisions of the neural folds. What he has termed the second brain vesicle is in reality the second division of the hindbrain.

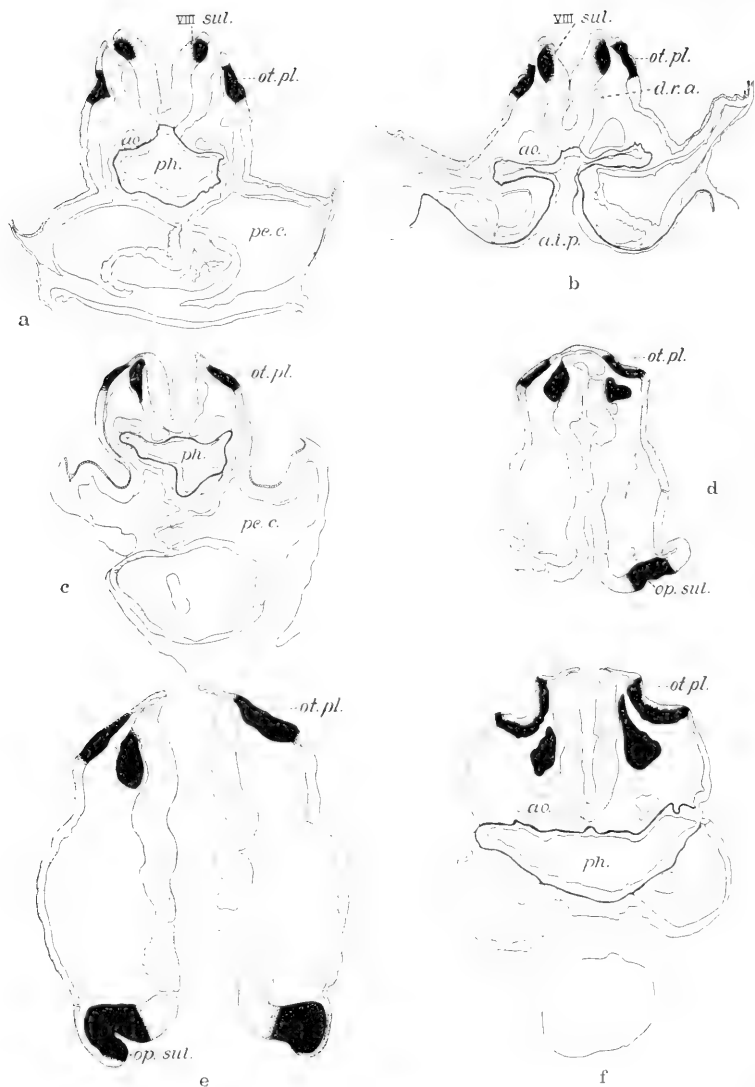


Fig. 2 A series of tracings made from the sections with the Edinger apparatus, $\times 200$, and reduced to $66\frac{2}{3}$ diameters in reproduction. The solid color marks the otic plate and the acousticofacial ganglion and optic anlage; the rest of the nervous system, including the neural crest and the ectoderm, are stippled, the outer boundary of the pharynx is indicated by a heavy line and the myoepicardium marked by horizontal lines. The mesenchyme is not shown except where it was shrunken and then its outer limits are drawn. a. Mall embryo no. 391—eight somites—section 45. b. HS7—eight somites—section 64 (cf. figs. 3 and 4). c. Eternod 'Du Ga'—nine somites—section 49. d. N.Y.U. embryo no. 4—fourteen somites—section 32. e. Pfannenstiel III—fourteen somites—section 41. f. Mall embryo no. 470—sixteen somites—sl. 2-1-7. *a.i.p.*, anterior intestinal portal; *ao.*, aorta; *d.r.v.*, dorsal second aortic ramus; *op.sul.*, optic sulcus; *pe.c.*, pericardial cavity; *ph.*, pharynx; VIII sul.; otic sulcus. In this and all other figures of sections the right side of the embryo appears at the observer's left.

Here we find the summit of the cranial flexure marked by a boss, the midbrain, *m.b.*, limited fore and aft by slight constrictions of the neural folds. Caudal to it the two hindbrain subdivisions are obvious. Cross-section pictures of the otic region

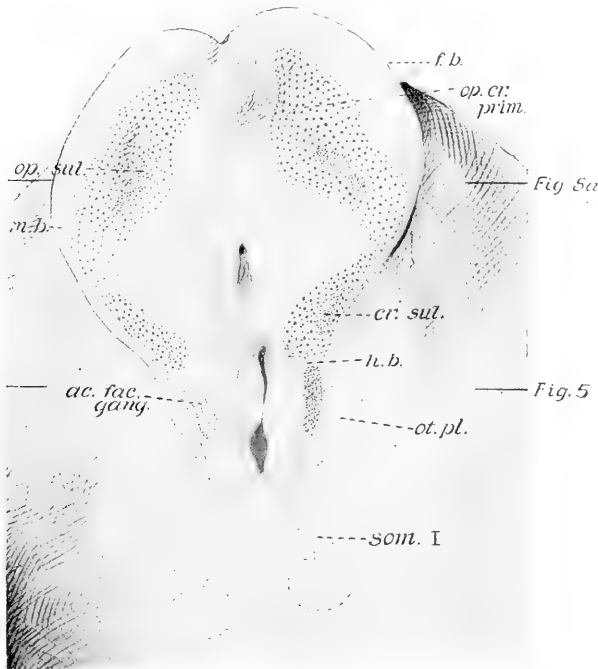


Fig. 3 A geometric projection of the head end of a model of H87 (Univ. of Chicago Coll.), drawn by J. F. Didusch and reduced to 100 diameters. Upon it the otic and optic-crest primordia are plotted and represented by stippling. The densest areas indicate the position of sulci; *ac. fac. gang.*, acousticofacial ganglion; *cr. sul.*, neural-crest sulcus corresponding in this case to the position of the future Vth ganglion; *f.b.*, forebrain; *h.b.*, hindbrain; *m.b.*, midbrain; *op.cr.prim.*, optic-crest primordium; *ot.pl.*, otic plate; *som. I*, first somite.

are given in figures 5 and 2b which are taken from successive sections, the level of which is indicated in the two general views. The photomicrograph (fig. 5) brings out clearly the apparent evagination of the neural fold at the site of the ganglionic anlage, an earlier stage of which we saw in the eight-somite embryo

of the Carnegie Collection (Dandy) in figure 2a. No actual evagination occurs, however, as we shall see: the sulcus is merely an effect of the lateral migration of the ganglionic cells en masse. Caudally they are already more loosely arranged than they appear in the figures and the epithelial arrangement which characterizes the neural fold is disappearing. The delaminating mass is, however, differentiated from the surrounding mesen-

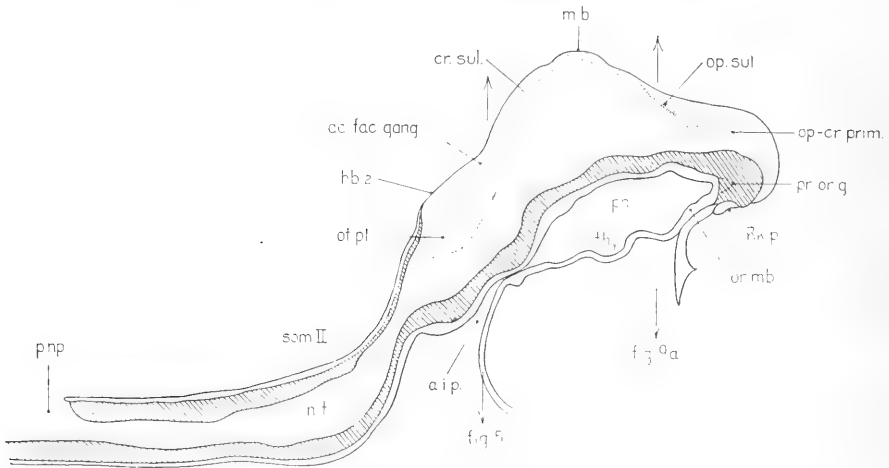


Fig. 4 A projection reconstruction of the medial aspect of H87 as if cut in the midsagittal plane. Only nervous system and gut are represented. The cut surfaces are indicated by hatching. The primordia are indicated as in figure 3. *a.i.p.*, anterior intestinal portal; *h.b.2*, second subdivision of the hindbrain; *N.T.*, closed region of neural tube; *or.memb.*, oral membrane; *ph.*, pharynx; *p.n.p.*, posterior neuropore; *pr.or.g.*, preoral gut; *Rk.p.*, Rathke's pouch; *som.II*, these letters indicate position and extent of second somite; *thy.*, thyroid evagination. Other abbreviations as in figure 3. $\times 120$.

chyme by the closer proximity of the cells to one another and by their deeper stain.

At the caudal end of the anlage it is possible that some cells are migrating out into the mesenchyme, but the plane of section is oblique, making the microscopic picture difficult to interpret.

In the next embryo, 'Du Ga' of Eternod, we find a great increase in the cephalic mesenchyme; the head and especially the first two visceral arches are taking form. The neural folds have

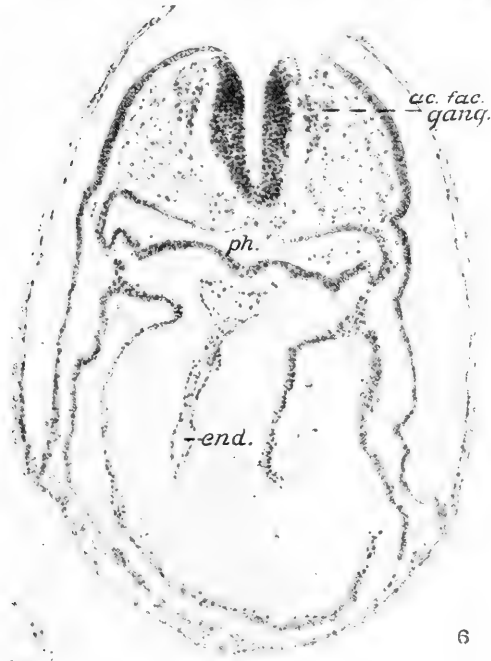
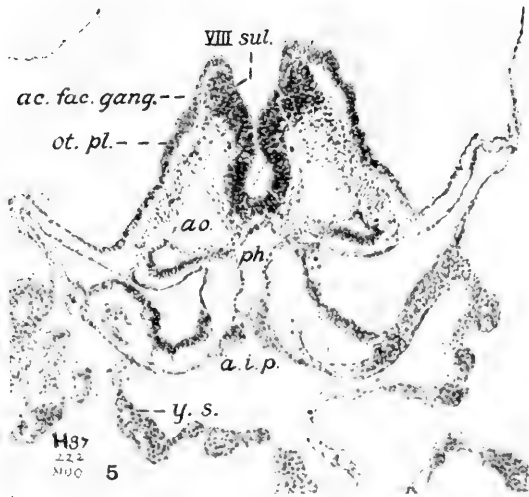


Fig. 5 A photomicrograph of section 63 from the eight-somite embryo H87. $\times 100$. The plane of section passes through the ganglionic anlage on both sides and through the rostral end of the otic plate. *ac. fac. gang.*, acousticofacial ganglion; *a.i.p.*, anterior intestinal portal; *ao.*, aorta; *ot. pl.*, otic plate; *ph.*, pharynx; *y.s.*, yolk sac; *VIII sul.*, ganglionic sulcus.

Fig. 6 A photomicrograph of section 50 from the eleven-somite embryo H392 (Univ. of Chicago Coll.). The plane of section passes in front of the otic plate and shows the ganglia separated from the neural folds except at the dorsal edges. The tube is closed immediately caudal to this level. *end.*, cardiac endothelium.

closed at the caudal levels of the otic plate, but are still open in the region of the acousticofacial ganglion. Figure 2c represents the relations in a section through the caudal end of the right ganglion which is no longer in direct continuity with the neural fold except along its dorsal edge. From this well-fixed specimen, as well as from H637 which is equally good histologically, and from the description of Veit ('18), it is obvious that there has been a loosening up of the ganglionic anlage followed by a separation of the entire mass from the neural fold so that it has delaminated before the two folds meet to form a tube.

A word should be said here concerning this interpretation of Veit's findings in his excellently preserved eight-somite embryo. His clear, accurate description and objective figures leave no doubt but that his caudale Ganglienleiste is what we interpret as the acousticofacial ganglion. Having but the one specimen at his disposal, Veit lacked the necessary data for identifying the subdivisions of the neural folds, and consequently his suggestions as to the fate of the Ganglienleisten are purely speculative.

Turning now to the otic plate, no hint of an otic pit is to be found in either our eight- or nine-somite stages, nor did Keibel and Elze ('08) find any in their nine-somite embryo (N T No. 4). In the eight-somite specimen (H87) the otic plate is distinguished by a lighter peripheral zone. An additional feature appears in 'Du Ga' (nine somites) where Doctor Evans noted a peripheral brush border on the otic plate when he studied the series in 1910. In this embryo the plate appears as a boss on the side of the head. When we come to the eleven-somite embryo, H637, we find the invagination just beginning. The histological appearance in this case is shown in figure 7, which represents a median section through this early otic plate. As we pass down from the dorsal ectoderm (from right to left in the figure), it will be seen that the pseudostratified condition of the ectoderm is preserved, but the distal moieties of the cells have elongated while the nuclei remained basal in position. Thus the clear peripheral zone is formed which characterizes the otic plate in all the older members of the series. When a particular cell begins to divide the nucleus migrates peripheralward and, as

the mitotic figure develops, most of the cytoplasm flows up around it. The resemblance of the dividing cells to the germinal cells of the central nervous system is obvious and they occupy the same relative position in the epithelium. The brush border appears over the central cells of the otic plate, and in this section resembles a band of short exceedingly delicate cilia. At other levels it looks more like a granular exudate. This is probably not due to inadequate fixation, as the specimen came from an unruptured tubal pregnancy with a strikingly normal appear-

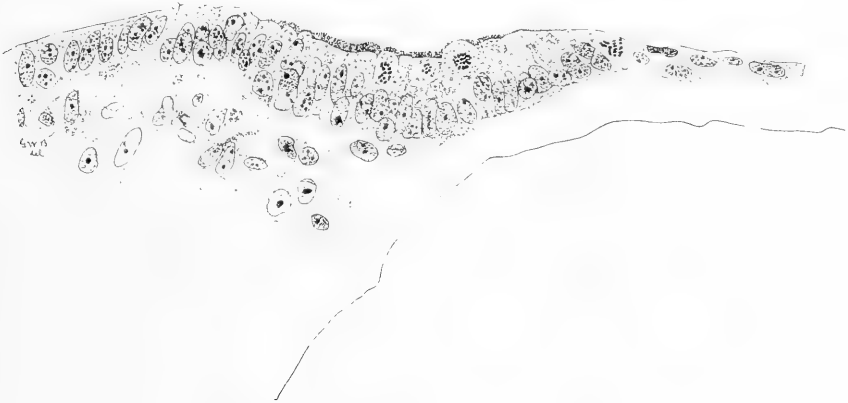


Fig. 7 A section through the middle of the otic plate of the eleven-somite embryo H637 (Univ. of Chicago Coll.). Camera drawing with 2-mm. Zeiss apochromate and comp. ocular 2 at 1000 diameters and reduced one-half in reproduction. The wavy line below at the right marks the external boundary of the neural tube. There is no mesenchyme between it and the otic plate.

ing implantation site and was immediately opened and placed in Zenker stock solution. That the embryo was normal is very probable. There were no immediate symptoms clinically except a lapsed menstrual period. Unfortunately for the embryo, it was cut and folded in the process of opening, but cytologically it is excellent. The plate cells show a well-developed internal reticular apparatus ('canalicular apparatus') which has not been detected in any other cells of the ectoderm at this stage. It appears as a series of clear spaces in the cytoplasm. It will be recalled that Ramón y Cajal ('12) found the internal reticular

apparatus to be the first cytoplasmic differentiation which appears in the histogenesis of the neuroblast.

The otic pit in the next two embryos is no further advanced in development than in that just described. In both it is shallow, in the eleven-somite H392 confined to the rostral end of the plate. The differentiation of the ganglion from the neural fold is complete histologically in H 197 (fig. 8) and figure 6 illustrates a slightly later stage in transverse section. In the fourteen-somite New York University embryo (Wallin) a greater portion of the plate is invaginating, as may be seen in figure 2d on the right (*ot. pl.*). The ganglion appears in this section and in the one shown in figure 9c. It is this which Wallin ('13) interpreted as the trigeminal ganglion, for he failed to recognize the otic plate. In Pfannenstiel III (fourteen somites) the otic pit has deepened and lies somewhat ventral of the center of the plate. The appearance in section may be seen in figures 2e and 9e; in the former the rostral end of the plate and the ganglion are shown on the left, while on the right the plane of section passes behind the ganglion. The section shown in figure 9e lies still further caudad and exhibits the progress made by the otic invagination in this embryo. Ectoderm and neural tube are perfectly distinct in both sections and were continuous across the midline in life, as is obvious from the study of a model constructed at a magnification of 200 diameters. The statement in the Normentafel indicates that Keibel and Elze ('08) also considered the neural tube closed in this region. Low ('08), however, was misled by the artificial breaking of the ectoderm and separation of the neural folds which extends far back into the region of the somites. Such cracks can be avoided, even in freshly preserved embryos, only by the most scrupulous care in dehydration and clearing if the material is to be imbedded in paraffin.

The otic plate of the fourteen-somite embryo H8 presents an intermediate stage between the last specimen and that shown in figure 2f. The whole plate is invaginating, but the pit is not so deep as it is in the sixteen-somite Mall embryo there figured. The marked increase in the thickness of the otic epithelium will be noted in this case. In both embryos the ganglion is almost

completely separated from the neural tube. This separation was apparently not so complete in the fifteen-somite embryo of Giglio-Tos ('02).

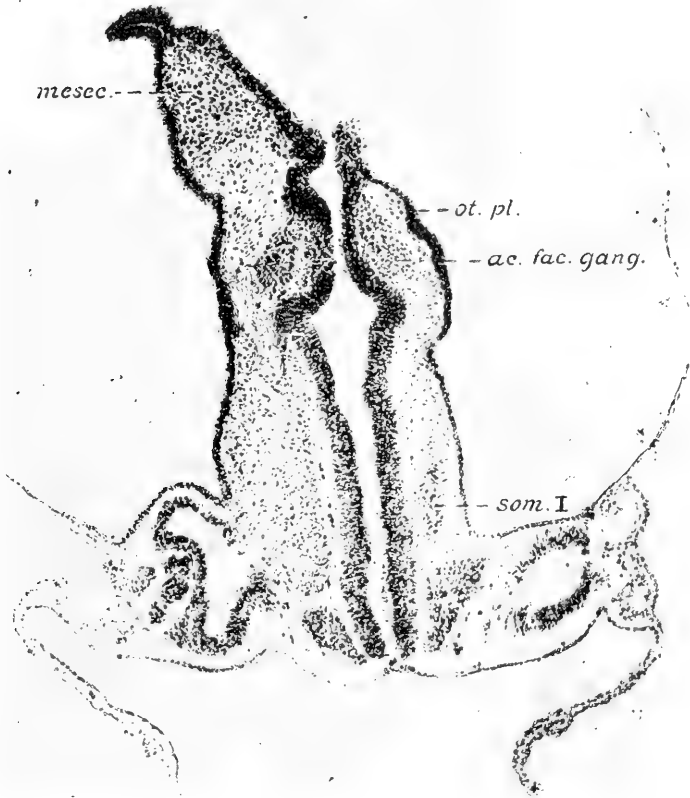


Fig. 8 A photomicrograph of the twenty-ninth section of the twelve-somite embryo H 197 (University of California Coll.). $\times 100$. The section passes horizontally through the hindbrain region. On the right of the figure the whole rostrocaudal extent of the otic plate appears with the beginning of the otic pit. The relations of the underlying acousticofacial ganglion (*ac. fac. gang.*) to it and to the neural folds are well shown. It will be seen that the neural fold is thinner where the ganglion has separated off.

Discussion—The otic primordia

The findings in respect to the otic plate require little comment. Wilson ('14) and Ingalls ('20) found it difficult to determine the limits of the plate in their two-to-three-somite specimens and this is true of all the earlier stages in its development. It is relatively more extensive shortly after it appears than it is later. There is at first an ill-defined thickening of the ectoderm opposite the second primary division of the hindbrain, but later while the ganglion is differentiating only the cells at the dorsal and rostral ends of the ectodermal area continue to elongate. The invagination begins at about the time the neural folds are closing at this level.

The history of the so-called acousticofacial ganglion has not been made out adequately in any mammal. It cannot be done in man until there is a complete series of well-preserved embryos available between fifteen- and twenty-five-somite stages. The conditions in this region of carnivore embryos seem to be quite similar to those in man. Weigner ('01) for the ferret and Schulte and Tilney ('15) for the cat consider that the geniculate and acoustic ganglia arise from a common anlage. It remains to be seen whether, in mammals as in many other vertebrates, the otic plate contributes cells to the eighth ganglion. Certainly, such an addition cannot be great. As for the geniculate ganglion, it is certain in the human that part of it arises from the epibranchial placode of the hyoid arch which is constantly present in fourteen-somite stages and later (cf. the findings of Giglio-Tos in his fifteen-somite specimen). This contribution from epibranchial placodes has been well established for all cranial nerves in Ichthyopsida which have a gustatory component, largely through the work of Landacre and his students. In the case of *Ameiurus*, Landacre ('10) found that the entire ninth ganglion arises from such a placode, and in the adult of this fish the ninth appears to be a pure gustatory nerve. The evidence here is so clear-cut as to warrant the working hypothesis that in other vertebrates the gustatory ganglion cells of the seventh, ninth, and tenth cranial nerves arise from epibranchial placodes. The

other components are probably derived from the neural crest, and there is abundant evidence in our series for such a proliferation at the levels corresponding to these nerves.

It should be said in conclusion that the acousticofacial ganglion in man is manifestly derived from the wall of the neural tube before its closure. The ganglion cells separate off, leaving the dorsal part of the fold thinner here than elsewhere as is well shown in figure 8. Even the few sections here figured leave no doubt of this. The evidence clearly excludes the possibility that the ganglion is formed from cells intermediate between neural and somatic ectoderm because the cells lying dorsal as well as medial to the ganglionic anlage enter into the formation of the definitive neural tube.

It is obvious from what has been said that the origin of the more rostral cranial ganglia in man must be studied largely in neural-fold stages. Much of the confusion in the literature is due to the failure to recognize this, to ignorance concerning the functional components of the cranial nerves, and to the fact that distinct components may have different origins.

THE OPTIC PRIMORDIA

The earliest anlage of the optic vesicle is so intimately associated with the cranial neural crest that the two must be discussed together. We will confine our attention to the extent and relations of the latter, as the details of this proliferation will be described elsewhere. The transformations which lead to the formation of the primary optic vesicle will be considered after we have sketched the development of the 'optic-crest primordium' as a whole.

The rostrally expanded neural folds in the younger members of the series show slight isolated thickenings which are difficult to interpret, if indeed they have any significance. When we come to the eight-somite embryo No. 391 (Dandy), we can identify definite primordia. Here, as we have seen, the otic plate and acousticofacial ganglion have appeared. Rostral to them we find the lateral third of each fold is thickened, the neural epithelium protrudes slightly toward the underlying mesenchyme and

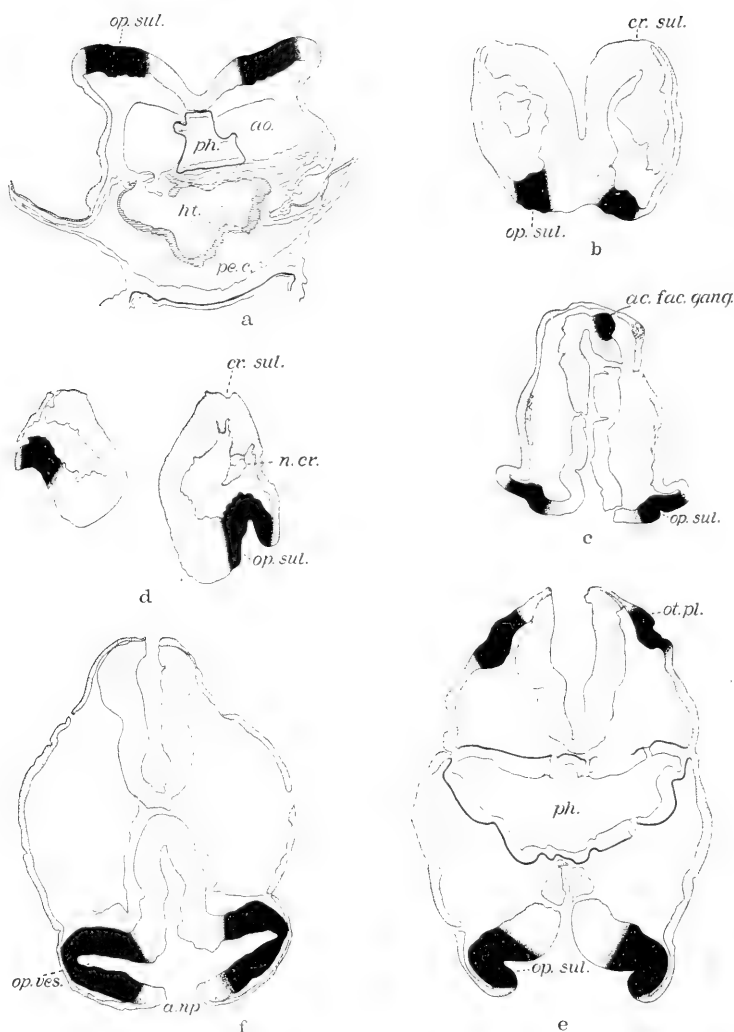


Fig. 9 A series to show the early development of the optic primordia corresponding to that of the otic anlagen. (See figure 2.) Here the optic primordium and otic anlagen are indicated by the solid color. a. H87—eight somites—section 28. b. Eternod 'Du Ga'—nine somites—section 12. c. Wallin embryo—fourteen somites—section 24. d. H392—eleven somites—section 9. e. Pfannenstiel III—fourteen somites—section 29. f. Mall no. 470—sixteen somites—slide 1-4-5.

in some sections there is a corresponding sulcus dorsally, i.e., on the future ventricular surface. On the left side three such thickenings can be recognized, one near the rostral end of the folds, one in the region of the cranial flexure, that is, in the mid-brain, and a third in the first hindbrain segment (cf. p. 218 *infra*). A few sections through the latter level show a further differentiation in that the thickened neural fold has no lower (external) boundary. It seems to merge with the mesenchyme beneath. As the later stages show, this is the very beginning of the neural-crest proliferation; in other words, there is an active migration of cells from the neural folds. The histologic picture in these particular sections is not above reproach, but under low powers of the microscope it presents the same appearance as do the perfectly preserved embryos of this period. These specimens make it clear also that we are not dealing in this case with an artefact. It is significant that the proliferation begins in the region where the semilunar ganglion can subsequently be identified.

In H87 (eight somites) it has been possible to study these primordia carefully, as the plane of section is particularly favorable. The forebrain here is in the form of a broad neural plate (fig. 3) which is in marked contrast with the domed folds of the four-somite H279, the Pfannenstiel-Krömer embryo ('Klb') and the eight-somite Dandy embryo. On the other hand, it resembles several embryos in this respect, e.g., the three-somite Carnegie No. 1878 (Ingalls), Wilson's 'H 98' (nine somites), H637 (eleven somites), the twelve-somite H 197, and the fourteen-somite N. Y. U. specimen (Wallin).

From the levels which show the pouch of Rathke [?] (fig. 4, *Rk. p.*) there are lateral thickenings of the folds extending caudally into the hindbrain, as is indicated by the stippling in figures 3 and 4 (*op. cr. prim.* and *cr.*). This longitudinal ridge may be divided into two parts, a larger broader area which converges rostrally toward its mate of the other side and a more caudal one which is narrower and more laterally situated. The constriction between them is in the region of the midbrain. This primordium gives rise not only to the optic vesicle, but to neural

crest as well; that is, to ganglion cells and head mesenchyme. Hence the somewhat labored term 'optic-crest primordium.' It is a continuous zone on the left side of the H87; on the right it is difficult to be certain of it in two adjacent sections through the midbrain, a distance of 16 μ . Brachet ('06, p. 244), in his amphibian studies, has pointed out the interesting and perhaps significant fact that the cranial neural crest and retina arise from homologous regions of the neural folds, and the continuity of the primordium in this series adds interest to our speculations on the subject.

As the figures show, the large rostral part of the primordium has a shallow sulcus on the upper, i.e., ventricular, surface which marks the position of the future optic evagination and is, in fact, the optic sulcus. As we approach the midbrain the primordium becomes narrower and involves the lateral part of the neural fold, and there is no sulcus (fig. 3). On the left at least the primordium continues into the hindbrain where cells are migrating out from it. Here, then, we have a typical neural crest. Some of these cells remain relatively closely packed and enter into the formation of the semilunar ganglion. Others become head mesenchyme. It is worthy of note, in view of the recent work of Landacre ('21), that promptly after the proliferation begins the mandibular and hyoid arches begin to take form (cf. the Ziegler model of Eternod's embryo 'Du Ga').

The appearance of a section through the rostral area of the primordium is shown in figure 9a, where both optic sulci appear. The primordium is indicated by the solid color. It is improbable that all of this eventually enters into the optic vesicle, as we shall see below.

It will be best to turn now to the eighth member of the series, the twelve-somite embryo H 197, because the plane of section in the forebrain is very like that of H87, whereas in the three intermediate stages it is less favorable. There is a great dorsal flexure in H 197 like that in the Wilson embryo H98 ('14) and consequently it was possible to obtain sections perpendicular rather than tangential to the surface of the forebrain folds. The optic-crest primordium shows several changes. Its rostral

division is wider, the optic sulcus has deepened so that the optic anlage is sharply delimited on all sides from the rest of the area. Mesial and rostral to it cells are migrating out from the primor-

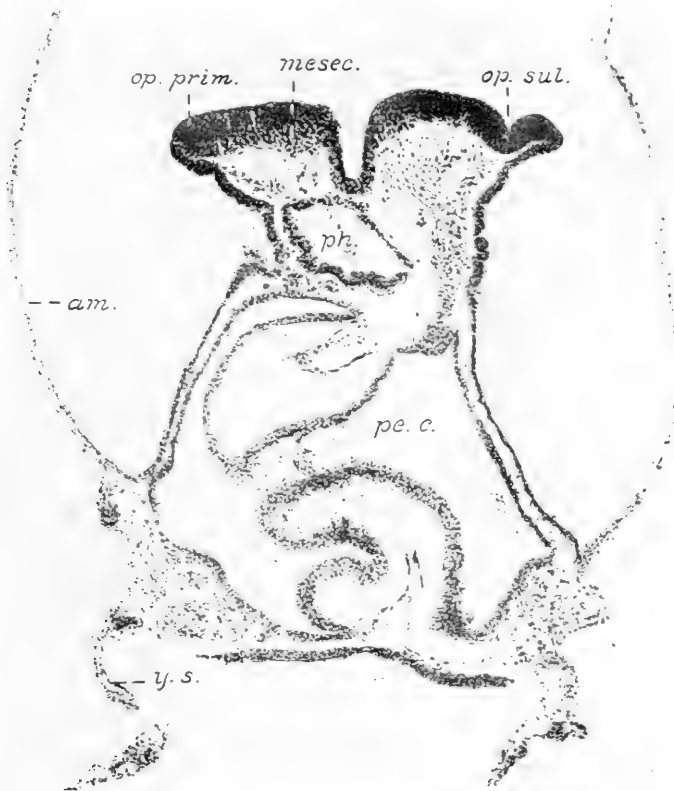


Fig. 10 A photomicrograph of the seventeenth section of the twelve-somite embryo H 197 (Univ. of California). $\times 100$. On the right the section passes near the middle of the optic anlage (*op.sul.*); on the left through its rostral end (*op.prim.*). Medial to the optic evagination the thickened neural fold is proliferating mesectoderm (*mesec.*). The section passes horizontally through the heart. *pe.c.*, pericardial cavity; *am.*, amnion; *ph.*, pharynx; *y.s.*, yolk sac.

dium as mesectoderm. Caudally the primordium continues without a break into the hindbrain where, as figure 8 (*mesec.*) shows, the mesectoderm is clearly differentiated from the rest of the mesenchyme by its deeper stain. The optic sulcus appears

on the right side of figure 10; on the left the section passes through the rostral end of the optic primordium (*op. prim.*). The more medial portion of the thickened primordium in this figure shows the indefinite lower boundary which is characteristic of a mesectodermal proliferation. It would seem, then, that the optic anlage arises only from the lateral part of the area indicated by stippling in figure 3, at levels corresponding to the caudal part of the forebrain, i.e., the future diencephalon.

H 197 is the only embryo we have seen which clearly shows mesectoderm formation in the forebrain, nor has it been observed in any other mammal. It can hardly be an abnormal activity in this particular case, for as a whole the embryo fits perfectly into the series. More than that, there are hints of such a process in another undoubtedly normal specimen, as we shall see. It is in better condition histologically than most young human embryos, as the photomicrographs (figs. 8 and 10) demonstrate.

The optic anlage of Eternod's nine-somite embryo 'Du Ga' (fig. 9b) is no farther along than in the eight-somite H87. In the eleven-somite H637 it is but little younger than in H 197 and the neural fold medial to it is greatly thickened. There are two spots where cells seem to be preparing to leave the primordium, and this evidence from an absolutely normal specimen affords the best evidence for regarding the extensive proliferation in H 197 as normal. Unfortunately, the mechanical injuries to the head in H 637 make modeling practically impossible. In the other embryo of this group, H392 (eleven somites), the optic sulcus is decidedly deeper than in H 197, as may be seen by comparing figures 9d and 10. Here, as in 'Du Ga,' the progress of the cranial flexure has bent the rostral end of the neural folds ventrally so that in the transverse series the first sections pass tangentially through the midbrain. Because of the oblique sections of the neural folds, it is impossible to get convincing pictures of neural-crest formation.

So far as the more caudal part of the optic-crest primordium is concerned, it is clear that in H637 (eleven somites) the optic anlage is continuous with the neural-crest proliferation of the mid- and hindbrain. In the beautiful 5μ sections of this series

one can observe every stage in the slipping out of the epithelial cells from the neural fold and Veit's ('18) excellent description of his 'craniale Ganglienleiste' can be verified in detail. In 'Du Ga' and H392 and the N. Y. U. No. 4 (fourteen somites) there is a continuous crest anlage in the midbrain and the pre-otic hindbrain. In the first it reaches almost to the acoustico-facial ganglion, while in H392 (eleven somites) it cannot be recognized below the trigeminal level.

Early history of optic vesicle

We may turn now to the mass movements in the rostral division of the anlage which produce the optic vesicle. The general features of the process may be gathered from a survey of the sections reproduced in figure 9. The first is taken from H87 (eight somites). On the left we have a slightly more caudal level than on the right as the head end curved somewhat to the left. The level of the section is indicated on figures 3 and 4. The thickening of the neural fold, viz., the optic anlage, is indicated by the solid color. The optic sulcus and the bulging of the anlage into the mesenchyme stand out clearly. From the lateral portion of it the optic vesicle will arise, as we have determined by the study of the corresponding region in the older embryos. This is an earlier stage of the optic primordium than has been described for any mammal.

Figure 9b is from the twelfth section of Eternod's nine-somite embryo 'Du Ga.' The rostral limb of the cranial flexure appears below; above is the caudal mesencephalic limb. Only the optic anlage is indicated by the solid color; it shows the characteristic thickening and the optic sulcus. Above in the figure is the mesectodermal proliferation with its corresponding ventricular sulcus. The optic anlagen in this specimen begin four sections (40μ) behind the rostral end of the neural folds and can be recognized in five or six sections.

The next stage we have is in the fourteen-somite embryo of the New York University collection. In this case the forebrain appears to have lagged behind the rest of the embryo in develop-

ment. Unlike the preceding specimen (fig. 9b) or the following one (fig. 9d), this has the anlagen cut transversely (fig. 9c). Active evagination has begun and on one side there is a deep pit at the center of the primordium, which is, however, confined to the single 5μ section here drawn. Figure 10 (*op. sul.*) shows the deepening optic sulcus as it appears in the twelve-somite embryo H 197. The optic portion is differentiated from the rest of the anlage as has been said and appears as a long narrow field measuring $60 \times 200\mu$ with the sulcus running through the middle. The optic anlage of the eleven-somite H637 is practically identical in appearance with this one.

Figure 9d was made from the ninth section of the eleven-somite embryo H392. The space separating the two neural folds here is the neural groove, the bottom of which is one-tenth of a millimeter caudalward in the series. The sulcus is cut obliquely, but is actually deeper than in any case we have considered. It will be noted that the optic evaginations are still directed ventrally. They correspond closely in position and extent to those of 'Du Ga,' in fact these two embryos are very similar in most respects.

Our next stage is found in the fourteen-somite embryo 'Pfst. III' (Keibel u. Elze, '08, Taf. 6), which is well known from the work of Low ('08) and others. This has the earliest optic anlage that has been described hitherto in man. It begins about 130μ behind the rostral tip of the nervous system and is present in twenty-one of the 10μ sections. Throughout this region the neural folds are still open. A section through the middle of the anlagen may be seen in figure 9e and a more caudal level appears in figure 2e. The striking change here is that the optic evaginations are now directed laterally toward the overlying ectoderm as a result of the rapid approximation of the neural folds. According to Low, there is an area of contact between the young optic vesicle and the overlying ectoderm. Keibel und Elze describe the vesicle as 'close' to the ectoderm, Bach und Seefelder ('14) intimate that there is no actual contact nor do Doctor Evans' tracings show any. Certainly, there is no mesoderm between the two epithelia and in the immediately following stages the lateral side of the vesicle comes into close contact with

the future lens epithelium. This is true of two embryos in the Carnegie Collection, No. 12 (fourteen somites) and No. 470 (sixteen somites). In the latter, as figure 9f shows, we have a fully formed optic vesicle resembling that of the His embryo 'EB' ('04) and of the twins described by Watts ('15). It is younger in the fourteen-somite embryo H8 in which the lumen is narrower and there is no ectodermal contact, although the anterior neuropore is as small as in the sixteen-somite No. 470.

Discussion—The optic vesicle

If we compare *e* and *f* of figure 9, it is clear that the optic anlage has begun to balloon out, by an interstitial growth as well as by a thinning of the wall. The optic sulcus has become V-shaped so that the anlage is a trough about as long at the base as it is deep. This holds not only for the fourteen-somite H8 and the sixteen-somite #470 (Carnegie coll.), but also for the embryos of Watts and that described by Bremer ('06). It is not until the twenty-three-somite stages that the vesicle has assumed a more or less spherical shape. It is difficult as yet to say how much of the area indicated by the solid color in figure 9e enters into the formation of the definitive optic vesicle. The narrow band of neural epithelium which separates vesicle and head ectoderm appears to grow very rapidly as the optic sulcus widens and deepens, and this growth certainly plays a part in the approximation of the neural folds. After their closure this lateralmost part of the original neural plate constitutes all there is of brain wall separating the two vesicles dorsally. It is of course possible that all of the dorsal and lateral diencephalic wall which we find between the optic stalks in later stages is derived from it. On the other hand, it seems more probable that, as the vesicle gradually pinches off, some of the original evagination is incorporated into the brain wall both dorsal as well as rostral and caudal to the developing optic stalk. Perhaps it would be better to say that a portion of the lateral brain wall is at first dragged out with the optic evagination. This would hold particularly for the zone between vesicle and head ectoderm. Schulte and Tilney ('15)

have presented strong evidence that this is so for the cat. On the basis of a complete series of models, they have described an absolute decrease in the size of the optic vesicle during the process of its separation from the brain and the formation of the stalk. It is not unlikely that the same conditions obtain in man. With the rapid enlargement of the optic ventricle the wall of the vesicle becomes thinner, whereas the diencephalic wall dorsal to it remains as thick as before.

These conditions emphasize another significant fact. The optic primordium is laterally placed, but not in direct continuity with the future skin ectoderm. The future roof plate and part of the alar plate intervene. This strongly supports the theory that the vertebrate eye originated within the central nervous system. Other evidence for this has been convincingly presented by Parker ('08). If the optic vesicle and its derivatives were lateral ectoderm incorporated into the neural plate, it would be necessary to assume that the hiatus left by the separation of the vesicle was filled by an ingrowth of neural epithelium from either side. Such ingrowth should be manifest first as a notch in the side of the anterior neuropore or later as a suture. There is no evidence of such conditions in the pertinent stages we have examined, and we may confidently say that mammalian ontogeny offers no support for the theory of the peripheral origin of the eye.

The anatomical evidence here, as in vertebrates generally, indicates that the optic primordia are lateral in position from the outset. The experimental evidence which has been well summarized by Mall ('19) is conflicting. The experiments of Stockard on *Amblystoma* ('14) are the most complete. In order to substantiate his theory of cyclopia, this investigator set out to prove that the earliest optic anlagen are median in position. In most of the embryos which survived the removal of the middle third of the rostral end of the neural plate the optic vesicles were subsequently lacking, whereas they were usually present when lateral moieties were extirpated. Stockard was convinced that the evidence demonstrated the existence of a median origin of the two subsequently lateral vesicles. It is possible that he did

not consider all the factors involved and that the results may be explained differently. It may be that his median extirpations had a much more general effect than he assumed and were in fact comparable to the general inhibition experiments with anaesthetics. The growing tip of the nervous system was removed, and this, in terms of Child's gradient hypothesis, is the dominant region of highest metabolism. According to the severity of the injury, the development of one or both eyes was more or less inhibited. The extirpations of lateral areas would be more convincing if there had been any attempt to map out the morphologically differentiated optic areas and remove them. Even then the regulatory restitution of an entire optic vesicle from a fragment of the primordium intrudes itself. It might prove possible to make small definitely localized injuries and trace them through their subsequent migrations, as Patterson ('10) did in his gastrulation experiments, and thus obtain more conclusive evidence.

COMPARATIVE DATA

From the comparative point of view, there are several interesting aspects of these observations. Man is the only vertebrate species on record in which the otic primordium appears before the optic. The otic plate can be recognized at an extraordinarily early period—earlier, in fact than in any other form for which we have accurate data. Conversely, the optic anlage is differentiated relatively later than in most other mammals, if we take into consideration the fact that the earliest stage described in the literature corresponds to our fourth stage (fig. 9d). The optic sulcus can be identified in man at a slightly earlier stage than that in which the otic plate appears in other mammals. The following résumé includes only the available mammalian literature.

Artiodactyls

Bonnet's ('01) account of early sheep embryos gives only enough to make it clear that the optic primordium precedes the otic in this form. Neither was identified in his twelve-somite

specimen (p. 10), but a fourteen-somite embryo (fig. 13) had well-developed optic vesicles, which indicates that the first anlage will be found at least as early as it is in the human. 'Das Ohrgrübchen' is first mentioned in a nineteen-somite stage.

The Normentafeln of Sakurai ('06) for the deer are more complete. The optic pit is first indicated in table 15 (eleven-somite embryo), while the otic plate appears at fourteen somites (table 17).

Keibel's first stage of the optic vesicle in the pig is a well-defined pit in the forebrain of a nine-somite embryo ('97, table 30). There is also the first hint of the otic plate here, but it must be remembered that the optic anlage doubtless appears earlier than this stage. The ten-somite series from which the Ziegler model was made has early optic vesicles rather than simple 'foveolae.' There is a marked evagination with a relatively wide lumen as yet directed ventrally, not laterally.

Carnivores

Weigner ('01) found the 'first signs' of an optic vesicle in ferret embryos 1.2 to 1.5 mm. in length in which the neural tube had not yet completely closed and the otic pits were already present. His data are not sufficiently exact to make it clear which primordium is actually the first to appear. In his careful study of a three-somite ferret embryo, Yeats ('11) refers to an 'optic prosomere,' and it would seem from his figures that there is an optic sulcus here. No mention is made of otic primordia.

There are two excellent papers on the early sensory anlagen in the cat. The earlier work of Martin ('90) can be best considered in the light of the more complete and thorough studies of Schulte and Tilney ('15). Here (p. 322) it is probable that the optic anlage was indicated in the three-somite embryo. The four-somite specimen had optic sulci which resemble those of man (fig. 9d) rather than the optic 'foveolae' of the pig. In both cases the neural folds were still open throughout their extent. In the older one they identified trigeminal and acousticofacial

ganglia, but the otic plate is not mentioned. It is possible that the latter appears early in the cat, although it certainly does not precede the optic anlage as in man.

The neural crest deserves further study in the carnivores. Weigner found no evidence for mesectoderm formation in the ferret and Schulte and Tilney affirm that all cells which leave the neural folds enter into the cranial ganglia. Martin (p. 342) found 'neural crest' beginning 'dicht hinter' the optic vesicle and extending through the midbrain giving rise to the sensory components of the third, fourth, and part of the fifth cranial nerves. If the microscopic picture in these forms is really not complicated by a mesectodermal proliferation, they are particularly favorable material for the study of the origin of the cranial ganglia and especially of such problems as the origin and fate of the muscle sense cells of the oculomotor nerves. On the other hand, it is also possible that the particular embryonic stages during which the mesectoderm migrates out have not yet been studied. It would seem that in the cat the nervous system is differentiating more rapidly than the axial mesoderm and that a close series, so far as the number of somites is concerned, may have distinct gaps in it. In man the period of pro-otic mesectoderm formation is limited to stages between eight and twelve somites, and it may therefore be that this phenomenon occurs between five- and seven-somite stages in the cat—a period which was not represented in the Columbia University series.

For the dog we have the descriptions of Bischoff ('45) and Bonnet ('01). Figure 36 of the former shows an embryo of about ten somites with obvious optic vesicles. The latter's figure 39 is taken from a section of an eight-to-nine-somite specimen and seems to pass through the edge of the optic primordium. Bonnet's ten-somite embryo has both optic vesicles and otic pits. Here, then, as in the cat the two primordia arise at about the same time, the optic, however, taking precedence.

Rodents

When one considers the large collections of rodent embryos in many embryological laboratories, it is surprising that so little

work has been done on the early development of the nervous system in these forms. The only complete study is the *Normentafel* of Minot and Taylor ('05) for the rabbit. In the first embryo of their series they noted the broad expansion of the neural folds rostrally and suggested that this might be the first indication of the optic anlage. This specimen had five somites. Since the eight-somite embryo (no. 4) had well-developed vesicles, it is probable that there were optic anlagen in the cephalic plate of the former. The thickening for the otic plate as well as the 'acoustico-facial ganglion' and the trigeminal ganglion were recognized in the nine-somite specimen of table 5.

Keibel, in 1889, figured the optic primordia of a guinea-pig embryo 16 days, 7 hours, old in sagittal section (fig. 44 and 45) and Foriep ('05, p. 157) has reproduced a photograph of a transverse section from a 3-mm. embryo. In both instances there is a deep broad pit situated laterally in the neural fold. Bischoff's monograph on the guinea-pig furnishes no data on this subject.

Insectivores

For the mole we have concrete data in the classic monograph of Heape ('87). He recognized the optic grooves in an embryo of three somites (stage E) where they are as well developed as in our eleven-somite H392 (fig. 9d). Heape calls attention to the very early appearance and typical form of the optic primordia in this form where the eye is subsequently degenerate. The otic plate is referred to in stage J when the optic vesicles are well developed and the neural folds are closed as far forward as the cranial flexure. His figure 25 is from a section of a fourteen-somite embryo in stage H and shows a slightly thickened otic plate.

Primates

Our knowledge concerning the pertinent stages of primates other than man is due entirely to Selenka and Huprecht. Selenka ('91, '00) obtained three embryos from the period we are considering. His figure of the *Hylobates* embryo 'Ab' ('00,

fig. 24) represents a dorsal view of a three-somite specimen, of which the rostral end of the neural plate resembles that of H87 (eight somites). It shows two well-marked converging grooves which may well be the optic sulci. The thirteen-to-fourteen-somite embryo of *Semnopithecus* ('Wa,' Selenka, '03, figs. 11 and 12) has large optic vesicles, but no otic plate is indicated. There is a deep otic pit in the twenty-three somite *Cercocebus* embryo, 'Ce,' however.

From the *Normentafeln* of *Tarsius* (Huprecht and Keibel, '07) it would seem that the eight-somite embryo (figs. 8 and 9) had optic Anlagen somewhat further advanced than those of our eight-somite embryo H87 (fig. 9a). The youngest stage in which the otic plate could be recognized is that of twelve somites (table 5), where the neural folds were closed in the region of the optic vesicles. Here, then, as in most other forms, the optic primordia precede the otic in development.

The scanty and inaccurate data of this survey emphasize the need of detailed studies of early mammalian embryos both on their own account and for the light they will throw on human development.

CONCLUSIONS

1. The earliest sensory anlage in man is the otic plate which can be recognized in an embryo of two to three somites as a diffuse thickening of ectoderm in the hindbrain region. A four-somite embryo shows the beginning of the associated acoustico-facial ganglion, though its fate is not yet completely known.

2. The ganglion arises near, but not exactly at the dorsal edge of the open neural fold and the outermost part of the apparent evagination delaminates from the fold before the process of tube formation is completed. It is clearly derived from the wall of the definitive neural tube.

3. The otic epithelium differentiates by an elongation of the distal ends of the cells and the appearance of a brush border. Between ten- and twelve-somite stages invagination begins and there is a deep otic pit at sixteen somites.

4. The otic plate has been identified earlier in man than in any other vertebrate of which we have accurate data, and in this form only does it precede the optic anlage.

5. Isolated thickenings (growth centers) of the cranial neural folds appear at a stage of seven to eight somites, which promptly fuse to form a continuous ridge, the 'optic-crest primordium.' An associated ventricular sulcus in the forebrain levels of the ridge indicates the position of the optic anlage. This is the earliest stage of this anlage which has been recognized in a mammal.

6. The non-optic part of this primordium proliferates mesectoderm and a large part of the trigeminal ganglion.

7. The optic anlage appears laterally in the neural fold, but between it and the ectoderm there is an intervening zone which gives rise to part of the alar and roof plates of the future diencephalon. The optic vesicle is therefore derived entirely from the central nervous system.

8. At a stage of sixteen somites the optic vesicle is in contact with the overlying ectoderm.

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Resumen por el autor, Davidson Black.

Los núcleos motores de los nervios cerebrales en la Filogenia. Un estudio de fenómeno de la neurobiotaxis.

IV. Aves

En la parte descriptiva de este trabajo el autor describe con detalle la morfología y relaciones de los núcleos motores de los nervios cerebrales de *Cacatua roseicapilla* y *Ciconia alba*, comparándolos con las condiciones observadas en otras aves, así como con las observadas en los reptiles e ictiopsidos. El plan de los núcleos motores cerebrales de las aves, si bien exhibe algunas variaciones importantes en las diferentes familias, es esencialmente el mismo en todas las formas observadas y característicamente diferente del que se observa en los otros grupos de los vertebrados.

La asociación de los núcleos motores del V y VII nervio y la situación de los grupos celulares faciales sobre, y más frecuentemente, delante del nivel de salida de su raíz motriz constituyé en las aves un carácter que se encuentra solamente entre los vertebrados en los ciclóstomos. Del mismo modo la asociación de los núcleos dorsales del glossofaríngeo y vago, que es característica de las aves, es un rasgo que solamente se encuentra entre los demás vertebrados en los petromizontes, mientras que en la diferenciación del complejo intermedio motor X-XII ocupan una posición única en el tipo de los vertebrados. La posible significación de estas particularidades en el plan de los núcleos motores del cerebro de las aves es objeto de discusión, aduciendo el autor nuevas pruebas que tienden a confirmar el concepto neurobiotactico de la emigración nuclear dentro del sistema nervioso central.

Translation by José F. Nonidez
Cornell Medical College, New York

THE MOTOR NUCLEI OF THE CEREBRAL NERVES IN
PHYLOGENY. A STUDY OF THE PHENOMENA
OF NEUROBIOTAXIS

IV. AVES

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SIXTEEN FIGURES

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INTRODUCTORY

The following paper on the avian cerebral motor nuclei constitutes part 4 of a study on the phylogeny of these nuclei in vertebrates. Reference to my earlier communications on this subject renders unnecessary a further explanatory statement (8, 9, 10).

Cacatua roseicapilla, which is here described in comparison with other avian forms, is a member of the subfamily *Cacatuinae* of the family *Psittacidae*, suborder *Psittaci* (Gadow, 26; Evans, 21). Its distribution is restricted to Australia, where it has a wide range from New South Wales to the north-coast region (Salvadori, 48, p. 132). Among the members of this interesting family the fleshy tongue with its intrinsic musculature is relatively highly developed and the intrinsic specialization of the syrinx is also well marked. In these respects *Cacatua* offers a marked contrast to *Ciconia alba*, which has been restudied in the present connection and in which the tongue is rudimentary and the syrinx of simple and almost primitive structure (Beddard, 4, p. 65, et. seq.).

The motor nuclei and roots have already been studied and reconstruction charts have been made in the following avian forms: *Columba*, *Ciconia*, and *Chrysomitris* (Kappers, 32); *Ciconia* (Kappers, 33 and 35); *Casuaris*, *Spheniscus*, and *Colymbus* (Kappers, 34). Of these, reconstruction charts of the last three are reproduced in the present paper in figure 16, page 260.

MOTOR ROOTS AND NUCLEI IN CACATUA AND CICONIA

Nerve XII

Among birds two nuclei are usually concerned in the origin of the fibers which combine to form the hypoglossal roots. These nuclei, which have been variously named by different authors, consist essentially of a more specialized dorsal cell group usually intimately associated with cells derived from the dorsal motor X column, and a less specialized ventral nucleus forming the rostral extremity of the cervical somatic motor column.¹ In the following description Kappers' term 'nucleus intermedius' (33) has been applied to the former complex, in which there is usually to be distinguished a visceral portion, the *pars vagi*, and a somatic portion, the *pars hypoglossi*. For the less specialized

¹ On the other hand, Kosaka and Yagita (38) have concluded from their investigations that no true hypoglossal fibers take their origin from the rostral extremity of the cervical motor column in the birds examined by them (*Columba*, *Gallus*, and *Anas*). This conclusion, however, does not appear to be correct.

ventral nucleus Brandis' term 'nucleus ventralis hypoglossi' (13) has been retained.²

In *Cacatua* the nucleus intermedius XII forms a well-marked column of large multipolar cells which rests throughout its extent upon the periphery of the periependymal gray matter and is at all levels quite sharply demarcated from the adjacent nucleus motorius dorsalis X (figs. 1 and 3). In the closed portion of the medulla it lies ventro-lateral to the central canal, there being in this form a relatively large amount of periependymal gray matter between the latter structure and the dorsum of the raphé (cf. figs. 1 and 2). In this character *Cacatua*, in common with other members of the parrot family, differs from *Ciconia* and most other birds (Brandis, l.c., p. 631). In the open portion of the medulla the nucleus intermedius XII occupies an analogous position beneath the floor of the fourth ventricle.

Except at its rostral extremity, the area of the nucleus intermedius XII in cross-section is considerably greater than that of the dorsal motor vagus column, while in total length the former nucleus exceeds the latter and extends caudally for some distance beyond it (fig. 16, D, p. 260). Around the nucleus and within its interstices, numerous very fine oblique and longitudinally arranged medullated fibers are to be seen which give to it a very characteristic stippled appearance on section. These strands constitute the *fibrae propriae nuclei hypoglossi* of Koch (37), to which Brandis has also drawn attention (l.c.).

The root fibers arising in the nucleus intermedius XII, which in *Cacatua* constitutes the chief source of the hypoglossal nerve, converge for the most part towards the ventro-medial margin of this cell group. Here they become combined to form well-marked nerve strands, which then pass obliquely ventral and lateral to reach the periphery approximately along the lateral border of the inferior olivary nucleus. As in other birds, none

² The term nucleus intermedius, pars hypoglossi (or simply nucleus intermedius XII) is synonymous with Brandis' (l.c.) hypoglossal portion of the common vago-hypoglossal nucleus. The nucleus ventralis XII is equivalent to the nucleus XII of Turner (50) and of Kappers (33, fig. 5) as well as to the latter author's 'Fortsetzung des Cervicalmarks' (9).

of the root fibers arising in this nucleus appear to cross the raphé to emerge with those of the contralateral nerve (cf. Brandis, l.c.).

The nucleus ventralis hypoglossi is of relatively slight importance in *Cacatua*, since in this form comparatively few fibers contributing to the hypoglossal nerve arise here. The nucleus lies within the gray reticulum of the cervical motor column, of which indeed it forms the most rostral portion. Some of the

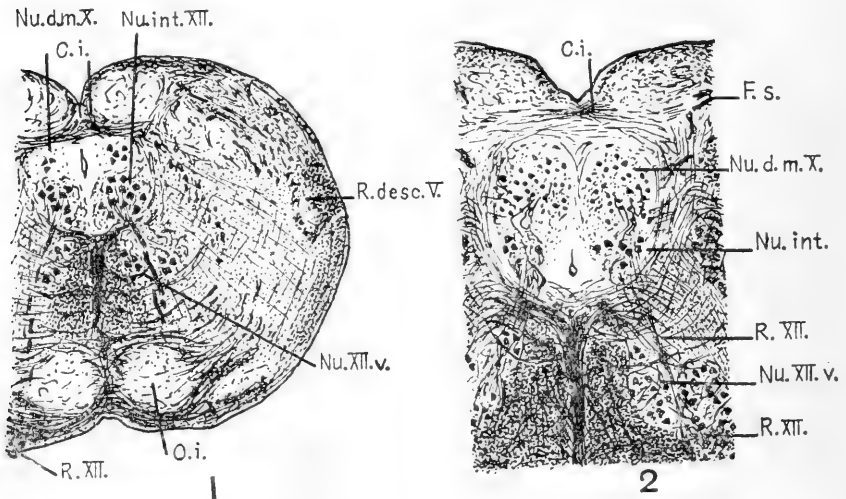


Fig. 1 *Cacatua roseicapilla*. Transverse section through the medulla just caudad of the calamus.

Fig. 2 *Ciconia alba*. Transverse section through the medulla caudad of the calamus. This drawing is at the same magnification as figure 1.

C.i., commissura infima (Bok, 11, p. 510); *F.s.*, fasciculus solitarius; *Nu.d.m. X.*, dorsal motor vagus nucleus; *Nu. int.*, nucleus intermedius X-XII; *Nu. int. XII.*, nucleus intermedius hypoglossi; *Nu. XII. v.*, ventral hypoglossal nucleus; *O.i.*, inferior olive; *R. desc. V.*, radix descendens trigemini; *R. XII.*, hypoglossal rootlets.

root fibers arising in this nucleus appear to cross the raphé and emerge by way of the contralateral hypoglossal nerve. Similar relations have been described in other birds by Brandis, who was able to confirm his observations by means of degeneration experiments.

In contrast to *Cacatua*, the majority of the fibers of the hypoglossal nerve in *Ciconia* arise from the nucleus ventralis XII.

In the latter animal this nucleus forms a slim well marked cell column which extends rostral from the cervical motor column into the medulla for some distance above the exit level of the first hypoglossal rootlet (fig. 16 C, p. 260). Caudally the nucleus ventralis occupies a dorso-medial position within the gray reticulum of the cervical motor column, in which its identity becomes gradually lost. Undoubtedly some of the root fibers arising in this nucleus present a crossed relationship.

As in *Cacatua*, the nucleus intermedius in *Ciconia* may be readily distinguished throughout its course from the ventrally situated cell clusters constituting the nucleus ventralis XII. In *Ciconia*, however, the nucleus intermedius is not a nucleus of origin for hypoglossal rootlets only, but, on the contrary, it is a cell complex in which arise both vagal and hypoglossal fibers. In this form, therefore, we have to distinguish two component cell groups in the nucleus intermedius, viz., the pars hypoglossi (nucleus intermedius XII) and the pars vagi (nucleus intermedius X) (fig. 2).³ Though intimately associated, the courses of their root fibers enable the limits of these two nuclear components of the intermedius cell group to be distinguished with considerable accuracy, and for this reason they have been plotted as separate entities on the reconstruction chart (fig. 16 C, p. 260).

In *Ciconia* comparatively few of the fibers composing the hypoglossal nerve have their origin in the cells of the nucleus intermedius XII and the cross-sectional area of the entire intermedius cell complex is at all levels much less than that of the adjacent dorsal motor vagus column.

In these characters *Ciconia* presents a marked contrast to *Cacatua*. On the other hand, the hypoglossal origin in *Ciconia* closely resembles that described by Kappers in *Colymbus*, *Casuaris*, and *Spheniscus* (34).

A third mode of hypoglossal origin has been described by Brandis and other investigators, who have shown that in some birds the XII nerve apparently may arise in its entirety from the rostral prolongation of the cervical motor column (nucleus

³ The latter term is synonymous with the Brandis vagal portion of the common vago-hypoglossal nucleus.

ventralis XII) in a manner very similar to the mode of origin of this nerve in many reptiles (fig. 15, p. 259).

On the basis of the current descriptions of the central origin of the hypoglossal nerve in birds, the forms thus far investigated may be arranged in three main groups, as follows:

Group I. Birds in which apparently the hypoglossal nerve arises wholly from the rostral prolongation of the cervical motor column: Lophortyx, Phasianus, Numida, Laurus, Anser, Phoenicopterus, Fulica (Brandis, 13).⁴

Group II. Birds in which the hypoglossus arises from both the nucleus intermedius XII and the nucleus ventralis XII: Anas, Gallus, Columba (Kosaka and Yagita, 38;⁵ Koch, 37; Brandis, 13); Casuaris, Spheniscus, Colymbus (Kappers, 34); Ciconia; Grus, Machetes, Falco, Struthio, Cairina, Corvus, and Passeres without exception, Cypselus (Brandis, l.c.); Gallus embryos (Bok, 11); Columba (Koch, 37; Turner, 50).⁶

Group III. Birds in which by far the greater part (or all?) of the hypoglossal nerve arises from the nucleus intermedius XII: The parrot family, among which two cockatoos (*Cacatua roseicapilla* and *C. galatea*) and two parakeets (*Melopsittacus* and *Palaeornis*) have been investigated (Brandis, l. c.).

Nerves IX, X, and XI

In *Cacatua*, as in all other birds examined, the dorsal motor vagus nucleus is continuous rostrally with that of the glossopharyngeus. These two nuclei together form the posterior visceral motor column, which in this form is relatively short. Though it extends rostral slightly above the exit level of the motor IX root, it is not prolonged far as a continuous cell column within the closed portion of the medulla and its caudal end falls some distance short of that of the nucleus intermedius XII (fig. 16 D, p. 260).

⁴ In the latter two, the flamingo and the coot, Brandis noted that the hypoglossal roots were small and the central nuclei poorly developed; and the origin of some of the nerve fibers from scattered cells representing a dorsal XII nucleus could not be excluded.

⁵ As already noted, these investigators did not consider that any true hypoglossal fibers had their origin in the cell column described above as the ventral XII nucleus (l.c., p. 167).

⁶ Turner, however, identified the nucleus intermedius XII as the spinal accessory nucleus.

From their nucleus of origin in the rostral end of the posterior visceral motor column the motor IX rootlets pass almost directly laterad and reach the periphery for the most part dorsal to the radix descendens trigemini (fig. 4).

The dorsal motor vagus nucleus forms a somewhat slim cell column which tapers towards its caudal end. In the latter situation the nuclei of either side lie dorso-lateral to the central canal and dorsal to the nucleus intermedius, being separated from one another by but a slight interval (fig. 1). Above the calamus these nuclei diverge from one another and lie within the gray matter of the ventricular floor as indicated in figure 3.

The most caudal motor X rootlets pass out almost directly dorsal, intermediate members of the series course dorso-lateral to reach the periphery, while the rootlets arising towards the rostral end of the nucleus emerge as do those of the motor IX nerve.

In *Cacatua roseicapilla* I have been unable to observe the origin of any undoubtedly motor vagus fibers from the cell column of the nucleus intermedius. In other words, no true nucleus intermedius X has been identified in this bird, though in the closely related form, *C. galatea*, Brandis (13) has described the origin of a few motor fibers from this source. In connection with this observation, however, the latter author noted that in parrots this cell group (which he termed the common X-XII nucleus) was almost wholly concerned in the supply of the hypoglossal nerve.

A small but evident ventro-lateral motor X nucleus is present in *Cacatua*. As in other birds this nucleus is not sharply circumscribed, and its loosely arranged groups of multipolar cells occupy a position lateral and somewhat ventral to the large nucleus intermedius XII. Kappers (l.c.) has already drawn attention to the presence of this nucleus in other avian forms (*Chrysomitris*, *Casuaris*, *Spheniscus*, *Colymbus*), in which he has definitely established its motor vagus character. Brandis also had earlier described this nucleus (12, pp. 182-3) and had figured it in the guinea-fowl (l.c., Taf. XIII, Fig. 5, *Numida*), but he failed to recognize its significance as a source for motor

X fibers. The nucleus in question is indicated on the reconstruction chart, but in view of the scattered arrangement of its elements it was not possible to mark definitely its rostral and caudal limits (fig. 16 D, p. 260).

In *Ciconia* the arrangement of the nuclei which go to make up the posterior visceral motor column is apparently more complicated than that observed in *Cacatua*. This is due to the presence

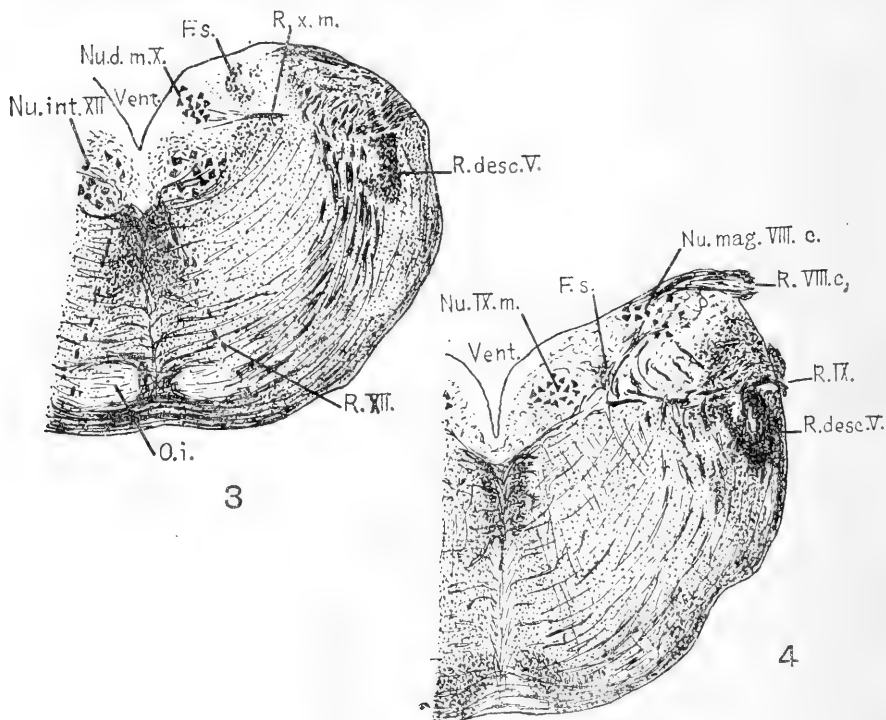


Fig. 3 *Cacatua roseicapilla*. Transverse section through the medulla a short distance rostral of the calamus.

Fig. 4 *Cacatua roseicapilla*. Transverse section through the medulla near the rostral end of the caudal visceral motor column. Figures 3 and 4 at same magnification as figure 1.

F.s., fasciculus solitarius; *Nu. d.m. X.*, dorsal motor vagus nucleus; *Nu. int. XII.*, nucleus intermedius hypoglossi; *Nu. mag. VIII.c.*, nucleus magnocellularis of the cochlear nerve (Brandis, 14; Holmes, 29); *Nu. IX m.*, motor glossopharyngeus nucleus; *O.i.*, inferior olive; *R. desc. V.*, radix descendens trigemini; *R. VIII. c.*, cochlear root fibers; *R. IX.*, glossopharyngeal root fibers; *R. XII.*, hypoglossal rootlets; *Vent.*, fourth ventricle.

of the nucleus intermedius X in relations that may be described as typically avian, since with but slight variation they have been found to obtain in the majority of the birds so far examined (fig. 16 A, B, C, and D, p. 260).

As in *Cacatua*, the most rostral portion of the dorsal part of the posterior visceral motor column in *Ciconia* is occupied by the motor IX nucleus. The relations of this nucleus and the mode of origin of its emergent roots in the latter form differ but little from those already described in *Cacatua* and require no further description here.

The dorsal motor vagus nucleus in *Ciconia* is much larger than in *Cacatua* and forms a conspicuous cell column which in the closed portion of the medulla occupies a position lateral and dorsal to the central canal within the periependymal gray and dorsal to the nucleus intermedius X and XII (fig. 2).

At the junction of the medulla and cord and below the caudal end of the nucleus intermedius X, the dorsal visceral motor cell column becomes reduced in bulk and its elements become arranged in irregularly spaced cell clusters.

In the same relative position the cell column, though discontinuous in places, was traced caudad a considerable distance. The origin of root fibers from the most caudal part of this nucleus was not demonstrated. The lower limit of the nucleus could not be definitely determined (fig. 16 C, p. 260).

The more caudal of the fine rootlets which arise from this cell column pass almost directly dorsal to reach the periphery, and in this respect resemble emergent XI nerve roots (cf. Lubosch, 41). The topography of the roots and nucleus of the latter nerve was not satisfactorily determined in *Cacatua* nor in *Ciconia*, but in neither of these forms does it appear that the dorsal motor vagus nucleus becomes continuous with the accessory nucleus in a manner similar to that observed in reptiles (10), amphibians (9), and selachians (8).

In *Ciconia* the nucleus intermedius X begins a short distance caudad of the exit level of the motor IX root as a ventral enlargement of the dorsal motor X column. From the outset, however, the morphology of its cells serves to differentiate it from the latter

nucleus. Over the greater portion of its extent it is intimately associated with the cells of the nucleus intermedius XII, the two nuclei forming a complex (fig. 2), the limits of whose component parts may, however, be distinguished by the course and distribution of their respective root fibers. In contrast to *Cacatua*, the cross-sectional area of the nucleus intermedius complex (combined X-XII components) in *Ciconia* is at all levels less than that of the adjacent dorsal motor vagus nucleus. The nucleus intermedius X is somewhat more extensive than the nucleus intermedius XII, so that it overlaps the latter nucleus both rostrally and caudally. In transverse sections of the brain stem at these levels the nucleus intermedius is formed wholly of motor vagus cells.

A ventro-lateral motor vagus nucleus may be clearly distinguished in *Ciconia* occupying a position analogous to that of the similarly named nucleus in *Cacatua*. As in the latter form, its rostral and caudal limits could not be accurately defined, though the position of its chief cell mass is indicated in the reconstruction chart, figure 16 C, page 260.

It is evident that the same grouping of avian forms will result from an arrangement on the basis of the vagal connections of the nucleus intermedius as on the basis of the central origin of the hypoglossal nerve. Thus, group I, as noted above, contains those forms in which the nucleus intermedius is largely vagal; group II is composed of forms in which the vagal and hypoglossal components of the nucleus intermedius are both quite evident, and group III consists of those forms in which the nucleus intermedius is to a very large extent a hypoglossal nucleus.

Nerve VII

The motor VII nerve in *Cacatua* has its origin within the brain stem from two distinct cell groups, which from their relative positions are termed, respectively, the dorsal and the ventral motor facial nuclei.⁷

⁷ These nuclei were first charted and fully described by Kappers (32), though this author noted at the time (l.c., p. 69) that two such nuclei had been observed

The dorsal motor VII nucleus occupies a central position within the formatio reticularis, and its entire bulk lies rostrad of the exit level of the motor VII root. At its rostral end this nucleus becomes continuous with a cell group which gives origin to a part of the motor V root, so that the cell column may be considered as a motor V-VII nuclear complex. Thus relations obtain here that are essentially similar to those of the V-VII motor nucleus in *Chrysomitris* which have been described by Kappers (32). At its caudal end this nucleus is somewhat enlarged and is prolonged ventrad to a level below that of the upper margin of the laterally situated nucleus olivaris superior.

The ventral motor VII nucleus in *Cacatua* is much smaller than the dorsal cell complex and lies very close to the ventro-lateral periphery of the medulla just medial to the nucleus olivaris superior (fig. 6). The sagittal relations of the two motor VII nuclei are indicated in the reconstruction chart, figure 16 D, page 260.

From their nuclei of origin the path of the emergent motor VII fibers to the periphery is indirect. From both dorsal and ventral cell groups these radicles pass dorso-medial and become collected in the dorsal tegmentum above and to the lateral side of the abducens nucleus, where their direction changes caudad and finally laterad to emerge on the lateral surface of the medulla for the most part dorsal to the radix descendens V, though numbers of the emergent VII fibers pierce the latter structure before making their exit (figs. 5 and 6).

The origin and relations of the motor VII nerve in *Ciconia* have already been described and figured by Kappers (32, figs. 46 and 69; 34, fig. 69), and in most essentials they agree with those in *Cacatua*. The dorsal motor VII cell group is, however, smaller in *Ciconia* than in *Cacatua*, and its cells do not become intermingled rostrally with motor V elements; while the ventral

by Wallenberg and had also been recorded by Kosaka and Hiraiwa (39) in *Gallus*. Indeed, the latter authors have described three facial nuclei in this bird: a chief or ventral nucleus, a dorsal or digastric nucleus, and a cell group associated with the latter and termed the 'Neben-kern.' It is highly probable that this 'Neben-kern' is represented in *Cacatua* by the caudo-ventral extension of the dorsal motor VII nucleus of this form (vide infra).

VII motor nucleus is larger in *Ciconia* and occupies a more caudal position than in *Cacatua* (cf. fig. 16 C and D, p. 260).

With the exception of *Casuaris*, in all the avian forms in which these relations have been carefully established (*Colomba*, *Chrysomitris*, *Ciconia*, *Spheniscus*, *Colymbus*, *Cacatua*) the motor VII nuclei are situated rostral to the exit level of the motor VII root. This relation, as Kappers has repeatedly noted, serves to distinguish these forms from all other vertebrates. In *Casuaris*, on the other hand, the motor nucleus lies on the level of its root exit in a relation somewhat similar to that obtaining in *Rana catésbiana* and *Petromyzon* (figs. 13, 14, 15, and 16).

Brandis (14) has recognized but one motor VII nucleus in each of the forms which he described, though Kappers' (32) subsequent careful investigations have shown that, at least in the case of *Columba*, Brandis' observations were in error. However, this does not serve to discount wholly the latter author's observations on the motor facial nucleus in other avian forms, since it is quite probable that not all birds possess two motor VII nuclei (e.g., *Casuaris*).

Nerve VI

The abducens nucleus in *Cacatua* forms a well-marked cell column of large multipolar cells which occupy a dorsal position in the *formatio reticularis*, to the lateral side of the *fasciculus longitudinalis medialis* (figs. 5 and 6). The nucleus lies almost wholly rostral of the exit level of the motor VII root, and extends from this level approximately to that of the caudal border of the motor V root (fig. 16 D, p. 260). The abducens roots are five in number and emerge in series on the periphery rostral of the exit level of the motor VII root.

In *Ciconia* the abducens nucleus occupies a somewhat more caudal position than in *Cacatua* and is considerably longer in rostro-caudal extent, though its position within the *formatio reticularis* in the two forms is similar. Eleven small emergent abducens rootlets were identified in *Ciconia*, all of which emerge in series rostral of the exit level of the motor VII root.

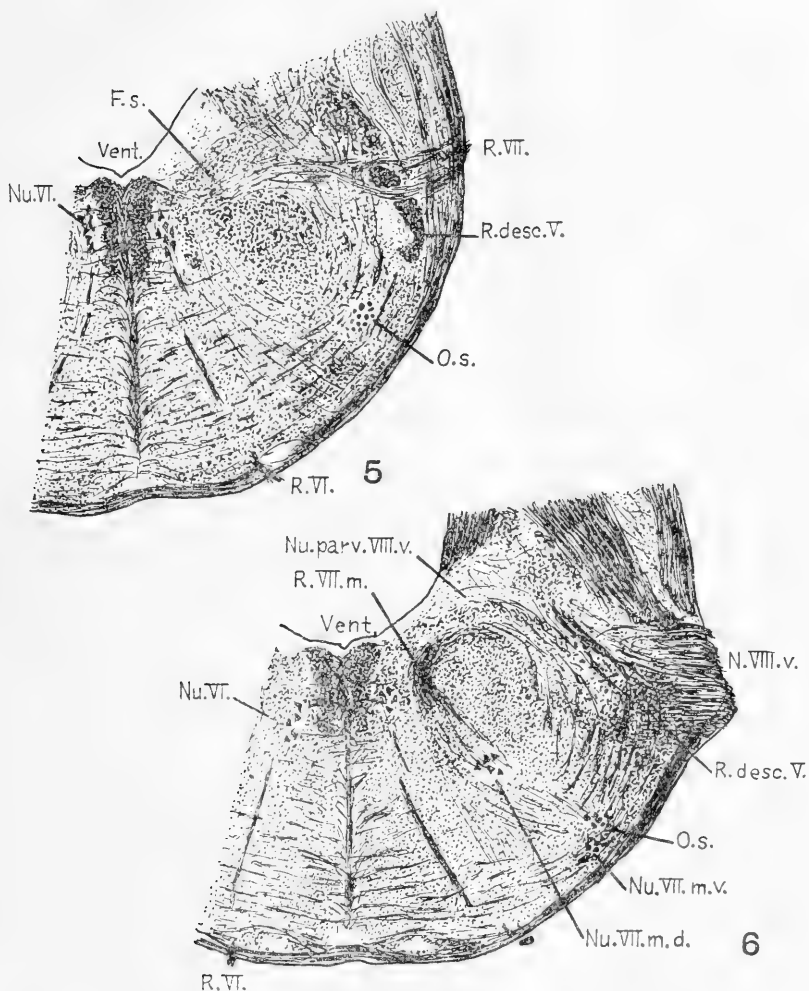


Fig. 5 *Cacatua roseicapilla*. Transverse section through the medulla at the exit level of the motor facial root. *R. VI*, fifth emergent abducens rootlet.

Fig. 6 *Cacatua roseicapilla*. Transverse section through the medulla at the exit level of the third abducens rootlet (*R. VI*). Figures 5 and 6 at the same magnification as figure 1.

F.s., fasciculus solitarius; *Nu. parv. VIII. v.*, small-celled vestibular nucleus (Brandis, 14); *Nu. VI.*, abducens nucleus; *Nu. VII. m.d.*, dorsal motor facial nucleus (= caudal part of motor V-VII nuclear complex); *Nu. VII. m.v.*, ventral motor facial nucleus; *N. VIII. v.*, vestibular root; *O.s.*, superior olive; *R. desc. V.*, radix descendens trigemini; *R. VII.*, root fibers of the facial nerve; *R. VII. m.*, intramedullary motor facial fibers; *Vent.*, fourth ventricle.

In *Cacatua*, *Ciconia*, *Colymbus*, and *Columba* the rootlets of the abducens nerve make their exit from the brain stem rostral of the exit level of the motor VII root in a manner similar to that obtaining among reptiles in *Boa* and *Varanus*. On the other hand, in *Spheniscus*, *Chrysomitris*, and *Casuaris*, some of the abducens rootlets emerge caudal to the lower border of the emergent motor VII root and resemble in this respect the mode of exit of the abducens rootlets in *Alligator* (figs. 15 and 16, pp. 259, 260).

Nerve V

In *Cacatua* the motor V nerve takes its origin within the brain stem from three quite distinct cell groups which are here distinguished as the dorsal motor V nucleus, the combined V-VII motor nucleus and the ventral or chief motor V nucleus.

The dorsal motor V nucleus forms a small circumscribed group of large multipolar cells situated beneath the gray matter of the ventricular floor and lateral to the fasciculus longitudinalis medialis. It is rostral of the abducens nucleus and is more dorso-laterally placed than the latter (cf. figs. 6 and 7). The radicular fibers arising from this cell group pass laterad and then ventro-laterad to emerge at the periphery, ventral to the entering sensory trigeminal root.

From the rostral end of the cell column whose relations as the dorsal VII motor nucleus have already been described, a number of radicular fibers emerge which take a characteristic indirect course dorso-medial through the tegmentum to join those arising from the dorsal motor V nucleus and to reach the periphery in common with the latter. The nucleus from which these fibers arise constitutes the combined V-VII cell group of the trigeminal motor nuclear complex, or the rostral continuation of the dorsal motor VII cell group of the facial motor complex.

The major part of the motor root of the trigeminal nerve in *Cacatua* arises in a large nucleus which is situated in the ventro-lateral area of the tegmentum, ventral to the emergent fibers arising in the dorsal motor V nucleus. From the ventral nucleus the emergent V root fibers pass directly ventro-lateral

and emerge on the periphery ventral to the entering sensory root of this nerve (fig. 7). At its dorso-medial angle the chief or ventral motor V nucleus comes into contact with the rostro-ventral border of the combined V-VII motor cell column (fig. 16 D, p. 260).

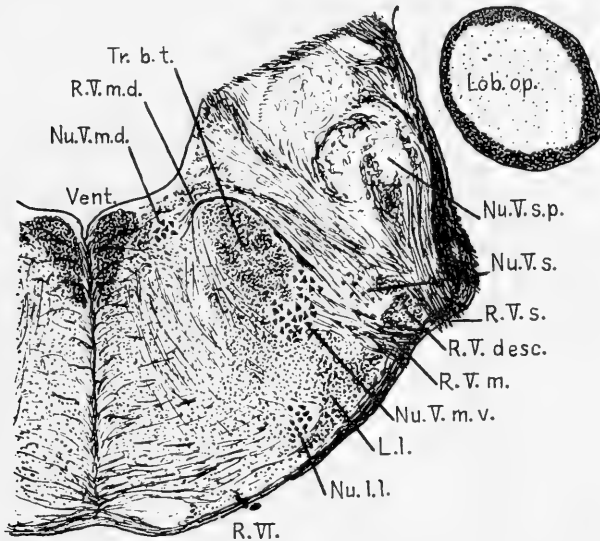


Fig. 7 *Cacatua roseicapilla*. Transverse section through the brain stem at the exit level of the motor trigeminal root and first abducens rootlet. Same magnification as figure 1. An absence of sharp radicular differentiation rendered obscure the relations of the mesencephalic V root at its exit level in *Cacatua*, a condition which van Valkenburg also found to obtain in both *Ciconia* and *Chrysomitris* (52 and 54). *L.l.*, secondary cochlear tract; *Lob. op.*, optic lobe; *Nu. l.l.*, nucleus of ascending cochlear tract; *Nu. V. m.d.*, dorsal motor V nucleus; *Nu. V. m.v.*, ventral or chief motor trigeminal nucleus; *Nu. V.s.*, sensory V nucleus with rostral end of the descending trigeminal root; *Nu. V.s.p.*, chief sensory trigeminal nucleus (cf. van Valkenburg, 53, figs. 7 and 8, Taf. XIX-XX, *Ciconia*); *R.V.desc.*, descending V sensory fibers; *R.V.m.*, motor trigeminal rootlets; *R.V.m.d.*, dorsal motor trigeminal rootlets; *R.V.s.*, entering sensory trigeminal fibers; *R.VI.*, first emergent abducens rootlet; *Tr. b.t.*, bulbo-tectal tract; *Vent.*, ventricular cavity.

The origin of the motor V root in *Ciconia* differs from that in *Cacatua* chiefly in the absence of fibers corresponding to those arising in the combined V-VII motor nucleus of the latter form. Motor trigeminal fibers were identified arising in a small

dorsal motor V nucleus in *Ciconia*, though in this animal by far the larger number arise in the chief or ventral motor V nucleus. The dorsal motor V nucleus has been observed in *Ciconia* by Kappers (32, 34), but at the time this investigator could not satisfy himself of its indubitable motor trigeminal character, and for this reason the nucleus in question was plotted in dotted lines on his original reconstruction charts.

An intermingling of motor V and VII nuclear elements has been observed in other avian forms by Kappers (l.c.). This author also noted that in some forms (e.g., *Spheniscus*, *Chrysomitris*, *Colymbus*) the ventral motor V nucleus was in reality a compound of two cell groups, the smaller lying medial to the larger. Further, it is probable that the V motor component of the combined V-VII motor column, when present, corresponds to the more medial of these motor trigeminal cell groups.

The origin of the motor V nerve in a number of other avian forms has been described in detail by Brandis (16). This author also distinguished three motor V cell groups which he termed the outer (ventral), the middle (which is continuous with the motor VII nucleus), and the inner (dorsal) motor V nuclei.⁸

In *Cacatua* the three motor trigeminal nuclei are situated approximately on the same level as their emergent root. This is true also to a large extent in *Columba*, *Chrysomitris*, and *Colymbus*. In *Ciconia*, on the other hand, a considerable portion of the motor V nuclear complex lies caudal of the motor V root exit level, while in *Spheniscus* the reverse condition obtains and the motor V nucleus is placed largely rostrad the level of its root exit. In all these forms the major portion of the motor trigeminal nucleus lies in a relatively ventral position within the tegmentum. In the latter respect the birds thus far examined differ markedly from *Casuaris*, in which the motor V nucleus occupies for the most part a dorsal position such as characterizes

⁸ In Turner's description (50), however, but two motor trigeminal nuclei were distinguished, viz., the lateral motor V nucleus corresponding to the chief or ventral motor V nucleus of the present description, and the deep motor V nucleus which would appear to be equivalent to the dorsal motor V nucleus described above.

this cell group among reptiles in chelonians and in Alligator (figs. 15 and 16, pp. 259, 260).

Nerves III and IV

The oculomotor nerve in *Cacatua* arises in a highly developed cell complex which, as in all other birds examined, is in close contiguity with the trochlear nucleus. Among birds the cell groups composing these associated nuclei are demarcated more clearly and precisely than is usual in any of the mammalian orders. This is largely due to the relatively slight development of small association elements in this region among birds.

The trochlear nucleus occupies a dorsal position, lying within the periependymal gray upon the dorsal surface of the fasciculus longitudinalis medialis (fig. 8). In its rostral portion the trochlear nucleus is accommodated within a distinct groove or excavation upon the dorsal surface of the latter fiber tract and a caudal extension of the pars dorsalis of the median oculomotor cell group comes to lie between the nucleus and the median sulcus of the ventricular floor (fig. 11, *h* and *i*).

The trochlear root fibers descend within the nucleus and towards the caudal end of the latter become collected into bundles which then emerge from its dorso-lateral margin. The radicular bundles thus formed pass dorso-lateral within the ventricular gray (fig. 8) to reach the superior medullary velum, in which the trochlear decussation occurs and from the lateral side of which the crossed trochlear roots emerge.

Within the oculomotor nuclear complex in *Cacatua* three well-marked subsidiary nuclei are to be distinguished which, in conformity with Kappers' descriptions (l.c.), are here termed the median, the dorso-lateral, and the accessory cell group, respectively. The general arrangement of these cell groups is illustrated in figure 9, where it will be seen that the median cell group is largest in cross-sectional area and is evidently divisible into dorsal and ventral portions. The largest elements within the oculomotor nuclear complex are found in the dorso-lateral cell group, while the smallest comprise the collection termed the

accessory cell group.⁹ Further, it will be evident that the trochlear nucleus at a more caudal level occupies a position analogous to that of the more rostrally situated dorso-lateral oculomotor cell group (fig. 9, *f*, *g*, and *h*). The practical absence of cells comparable to the numerous reticular elements characterizing this region in many mammals is a notable feature, though a well-

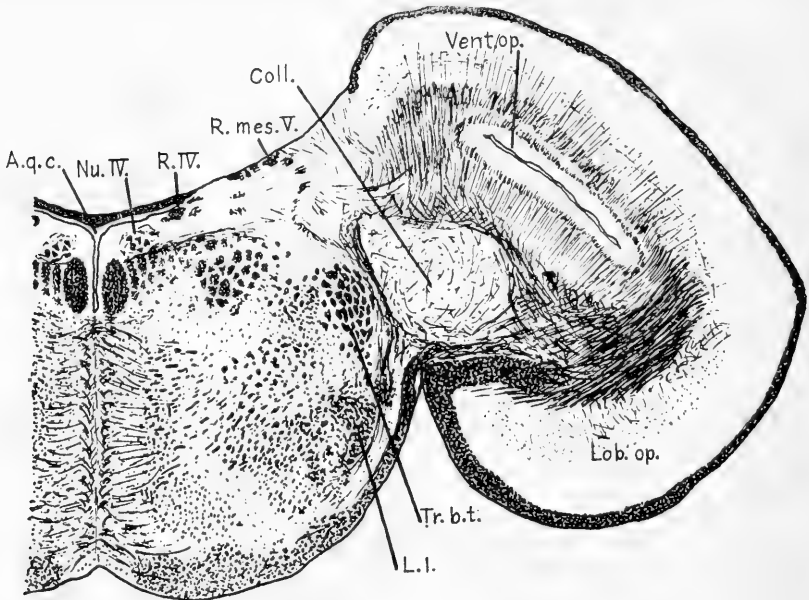


Fig. 8 *Cacatua roseicapilla*. Transverse section through the brain stem at the level of the junction of the middle and caudal thirds of the trochlear nucleus. Same magnification as figure 1. *Aq. c.*, aqueductus cerebri; *Coll.*, colliculus (= lateral mesencephalic ganglion of Wallenberg, 56); *L.I.*, secondary cochlear tract (Wallenberg, 58); *Lob. op.*, optic lobe; *Nu. IV.*, trochlear nucleus; *R. IV.*, homolateral trochlear root; *R. mes. V.*, mesencephalic trigeminus root (van Valkenburg, 52 and 54; Münzer u. Weiner, 43, Taf. VII); *Tr. b.t.*, tractus bulbotectalis; *Vent. op.*, optocoele.

⁹ There can be no doubt that, both in *Cacatua* and *Ciconia*, oculomotor fibers take their origin from the cells of this nucleus. The origin of such fibers from this nucleus in birds was first noted by Brandis (16), who termed the cell group in question the Edinger-Westphal nucleus. Subsequently these observations have been independently confirmed by Cajal and Kappers and more recently by Brouwer (17). Kappers' term accessory nucleus has been retained for convenience in the present description.

marked 'reticular' nucleus occurs within the ventricular gray below the level of the trochlear nucleus (fig. 9, *k*). The sagittal relations of these cell groups are shown in detail in the reconstruction chart (fig. 12 F, p. 254).

The oculomotor root fibers in *Cacatua* become collected on the ventral aspect of the nucleus (fig. 10) and then course



Fig. 9 *Cacatua roseicapilla*. Sketches a to k to illustrate the cellular detail at different representative levels from the rostral to the caudal end of the oculomotor-trochlear nuclear column (cf. reconstruction chart F, fig. 12, p. 254). *Nu. ac.*, accessory oculomotor cell group; *Nu. d.l.*, dorso-lateral oculomotor cell group; *Nu. m.d.*, dorsal part of the medial oculomotor cell group; *Nu. m.v.*, ventral part of the medial oculomotor cell group; *Nu. r.*, large cells on medial periphery of red nucleus; *Nu. ret.*, spindle-celled reticular cell column beginning caudal to the trochlear nucleus; *Nu. IV.*, trochlear nucleus; *R. IV.*, homolateral trochlear rootlets.

obliquely rostral and ventrad to emerge on the periphery as indicated in figure 11 (see also fig. 16 D, p. 260). Some of the fibers arising in the ventral part of the medial cell group undoubtedly decussate, as both Brandis and Kappers have observed to be the case in other avian forms.

The arrangement and differentiation of the oculomotor and trochlear nuclear elements in *Ciconia* is in general similar to that

obtaining in *Cacatua* and has already been described by Kappers in his earlier communications (Kappers, 34, figs. 73 and 76). The sagittal topography of this nuclear complex in *Ciconia* is

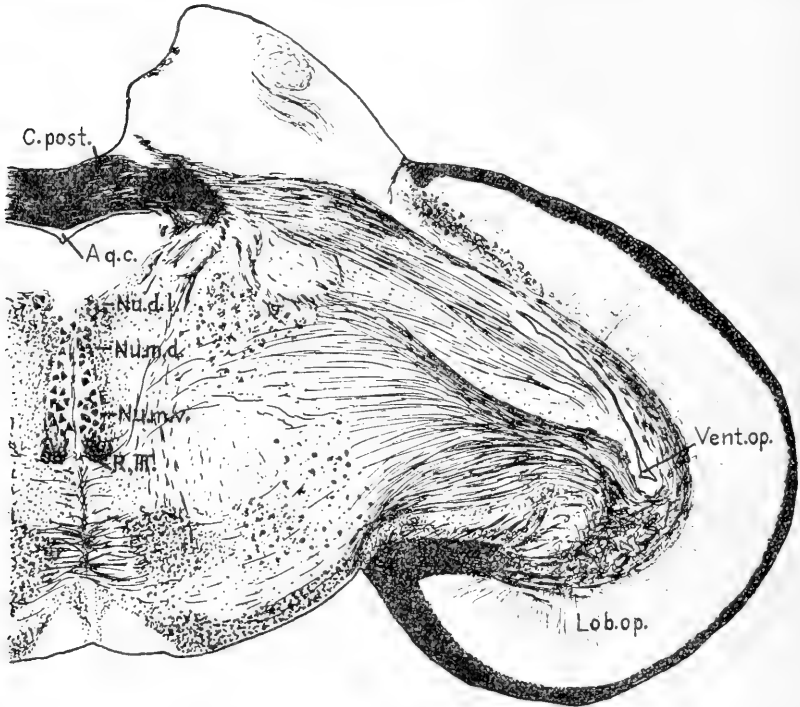


Fig. 10 *Cacatua roseicapilla*. Transverse section through the brain stem at the level of the junction of the rostral and middle thirds of the oculomotor nucleus. Same magnification as figure 1. *Aq. c.*, aqueductus cerebri; *C. post.*, posterior commissure; *Lob. op.*, optic lobe; *Nu. d.l.*, dorso-lateral oculomotor cell group; *Nu. m.d.*, dorsal part of the medial oculomotor cell group; *Nu. m.v.*, ventral part of the medial oculomotor cell group; *R.III.*, obliquely cut radicular fibers of the oculomotor nerve; *Vent. op.*, optocoele.

illustrated in the reconstruction chart, figure 12 E, page 254 (see also fig. 16 C, p. 260).

For accurate comparison of the details of the oculomotor and trochlear nuclear complex, reconstruction charts of these nuclei in *Cacatua* and *Ciconia* have been prepared on the same scale as Kappers' earlier charts of these structures in *Alligator*,

Varanus, Colymbus, and Spheniscus. The latter are here redrawn in figure 12 as mirror images of Kappers' earlier figures (34, figs. 60, 61, 77, and 78, respectively). The change from the original orientation has been made so that in these figures as in the larger

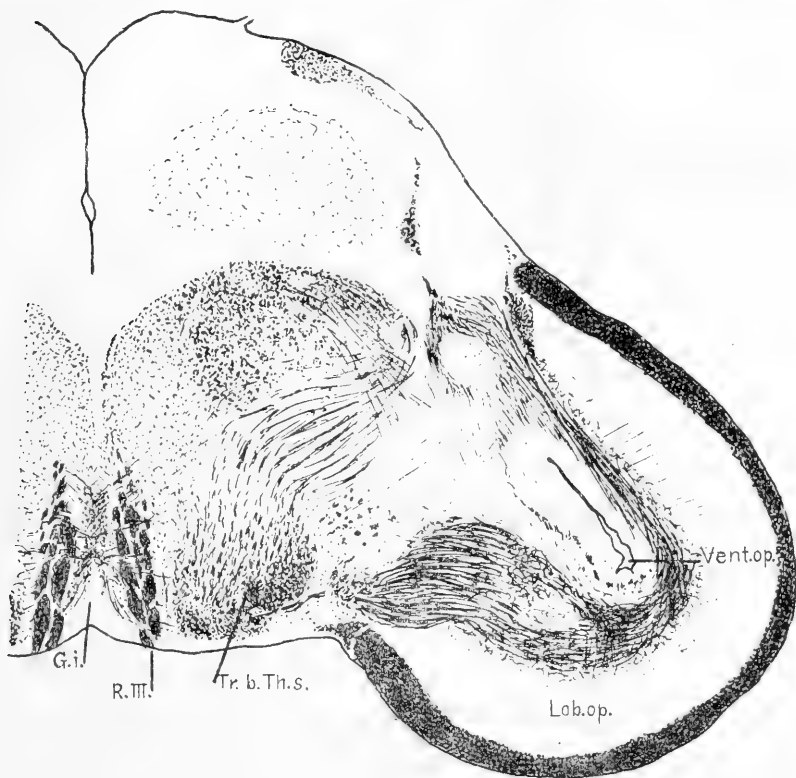
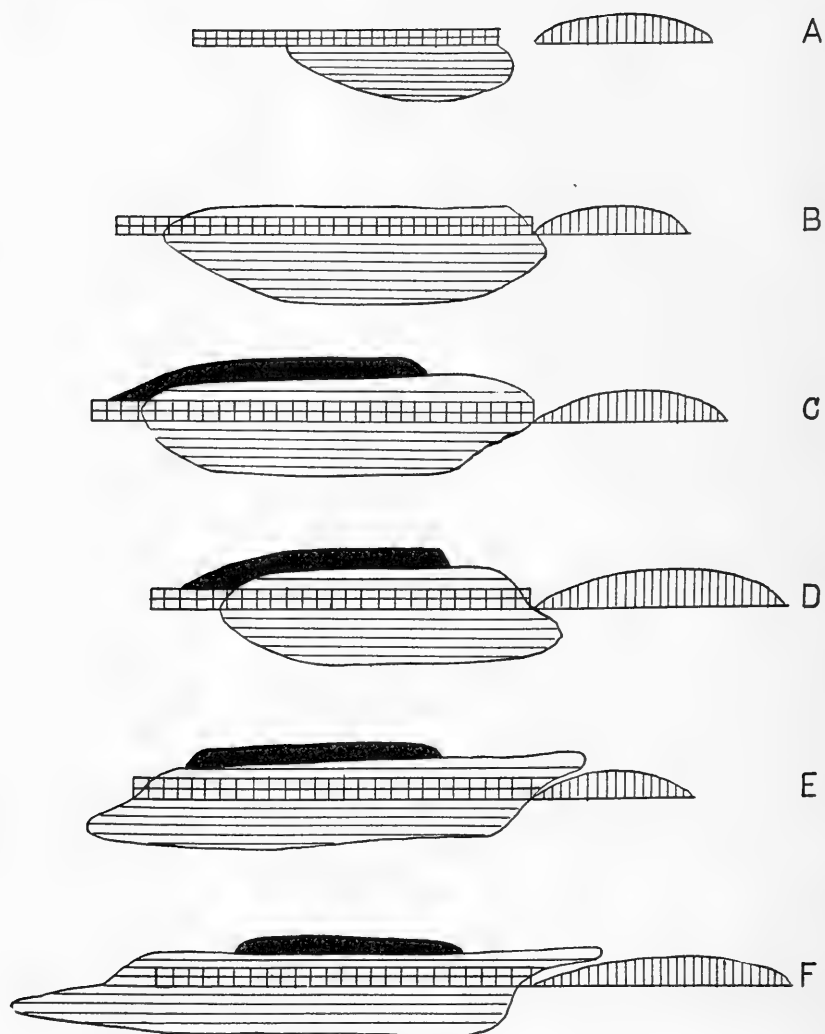


Fig. 11 *Cacatua roseicapilla*. Transverse section through the midbrain and thalamus at the exit level of the oculomotor roots. Same magnification as figure 1. *G.i.*, interpeduncular ganglion; *Lob. op.*, optic lobe; *R.III.*, emergent oculomotor root; *Tr. b. th.s.*, tractus bulbo-thalamicus (Wallenberg, 59) et bulbo-striaticus (Wallenberg, 57 and 59); *Vent. op.*, optocoele.

charts the rostral end of the reconstruction will be toward the left. To facilitate comparison, the charts in figure 12 are arranged so that the rostral end of the trochlear nucleus in all cases lies in the same vertical plane.



 Medial oculomotor cell group.

 Dorso-lateral oculomotor cell group.

 Nucleus accessorius III.

 Trochlear nucleus

It would appear from an examination of figure 12 that birds differ from the reptiles chiefly in the possession by the former of an accessory cell group of the oculomotor nucleus. This, however, is not entirely true, for Kappers (34, pp. 63-64) has found in one specimen of *Varanus* sp.? a cell group which in the morphology of its elements and in its general relations resembles closely the avian accessory oculomotor nucleus. No oculomotor fibers could be distinguished arising from this unique accessory cell group in *Varanus* sp.? and no similar cell group was observed in any other *Varanus* specimens nor in any other reptile examined.

Oculomotor nuclear differentiation in birds presents a marked advance over the condition obtaining in reptiles, especially in the differentiation of the median oculomotor cell group, since in none of the latter forms is a fully developed pars dorsalis of the median nucleus present.

From the data at hand it would seem that among birds all three divisions of the oculomotor nucleus are well differentiated and that contiguity of the trochlear nucleus with the dorso-lateral oculomotor cell group is a constant character. On the other hand, there would seem to be a considerable amount of variation characterizing the relations of the associated oculomotor and trochlear nuclei to the exit levels of the III and motor V roots, respectively.

Fig. 12 Reconstruction charts of the oculomotor and trochlear nuclei in sauropsidan forms. A, Alligator sclerops (after Kappers, 34, fig. 60); B, *Varanus salvator* (after Kappers, 34, fig. 61); C, *Spheniscus demersus* (after Kappers, 9, fig. 77); D, *Colymbus septentrionalis* (after Kappers, 34, fig. 78); E, *Ciconia alba*; F, *Cacatua roseicapilla*. The reconstructions A, B, C, and D have been redrawn as mirror images of Kappers' original charts, so that in the present paper both in these and in the other reconstructions the rostral end of the chart is toward the left side of the page. For comparison the drawings in this figure have been arranged so that the rostral ends of the trochlear nuclei are in line one below the other. See diagram above for explanation of signs. It is to be noted that no line of demarcation between the dorsal and ventral portions of the medial oculomotor nucleus is indicated on these reconstructions. The ventral part of the median nucleus in the charts lies below the lower border of the dorso-lateral column, while the dorsal portion is above this line (cf. fig. 9, p. 251).

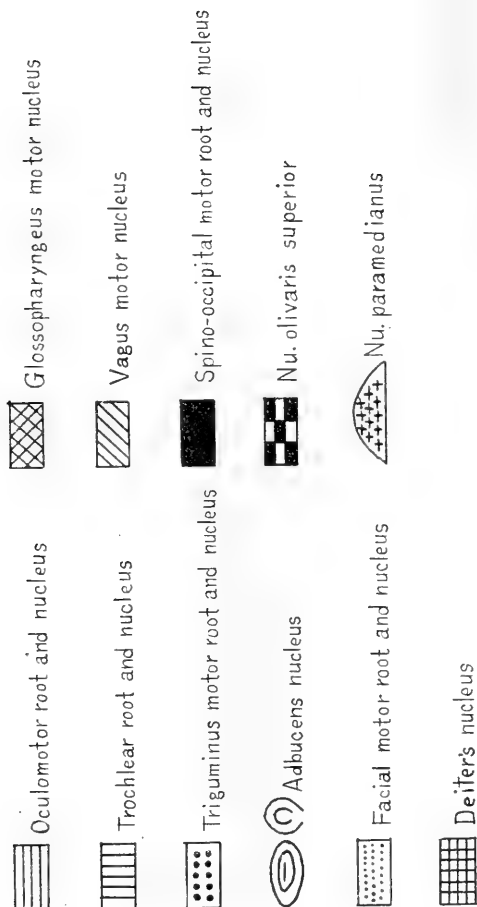
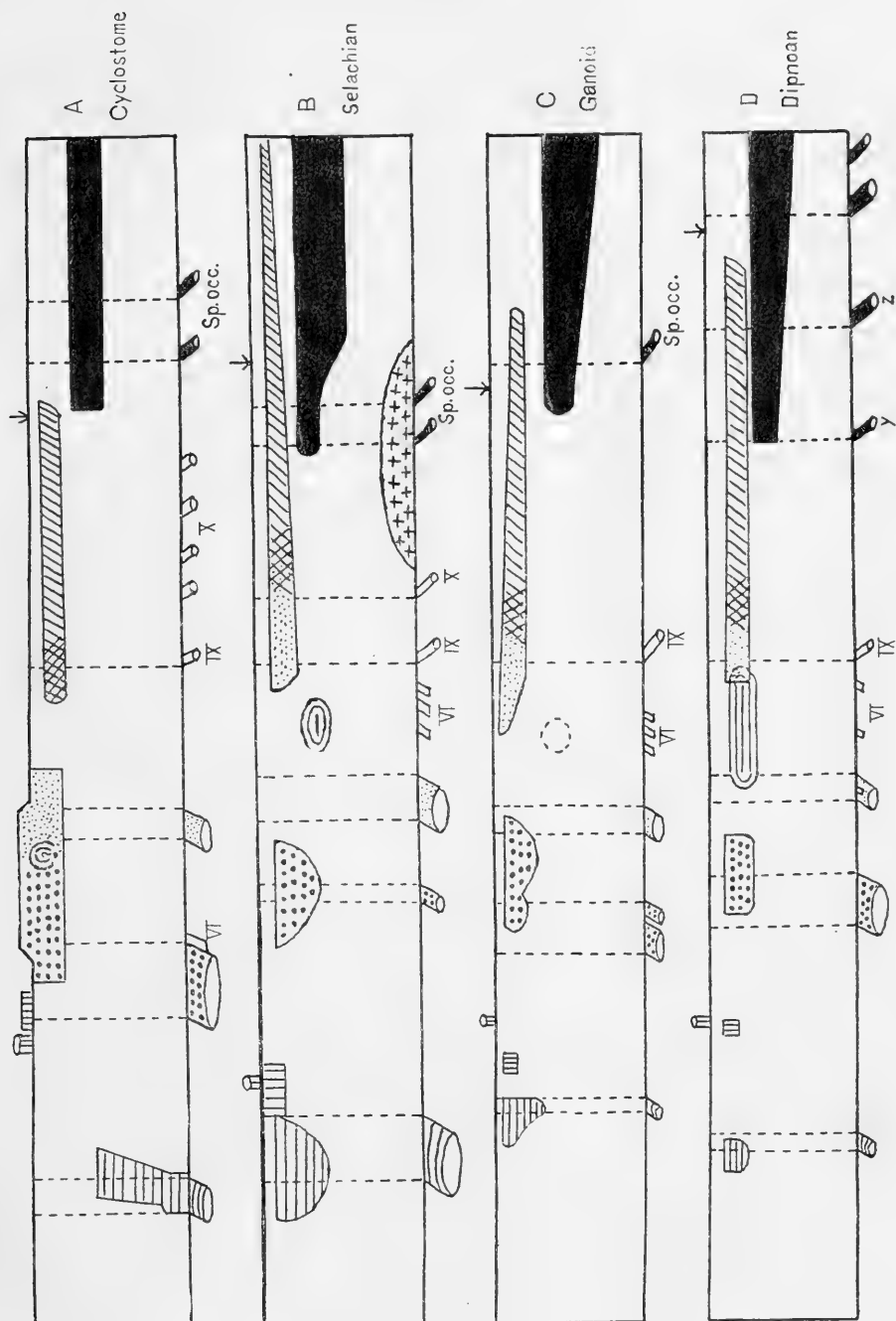


Fig. 13 Reconstruction charts of the motor roots and nuclei in lower ichthyopsidans for comparison with figures 14, 15 and 16. A, *Petromyzon fluviatilis* (after Kappers, 34); B, *Selache maxima* (S); C, *Polydon spathula* (S); D, *Neoceratodus forsteri* (after van der Horst, 51). Except for the caudal position of the trochlear nucleus in this form, the motor nuclear pattern in *Neoceratodus* is almost identical with that in the crossopterygians, *Polypterus* and *Calamoichthys* (van der Horst, l.c.) VI, abducens roots; IX, motor glossopharyngeal root; X, first motor vagus rootlet; y, z, and sp. occ., spino-occipital rootlets. The arrow indicates the site of the calamus. See diagram above for explanation of signs.



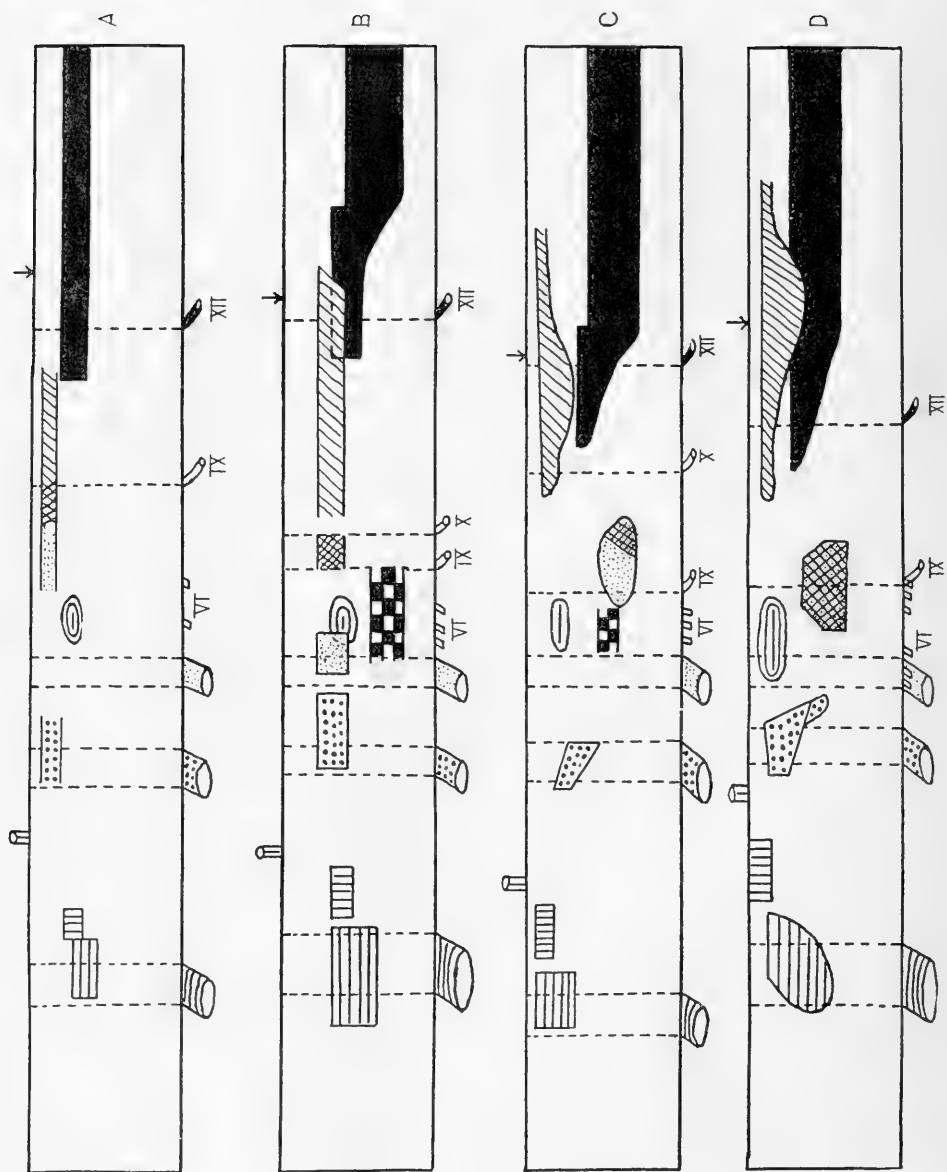


Fig. 14 Reconstruction charts of motor roots and nuclei. A, *Triton vulgaris* (after Kappers, 34); B, *Rana catesbiana* (9); C, *Damonia subtrijuga* (10); D, *Chelone mydas* (after Kappers, 34). XII, hypoglossal roots. Other signs and abbreviations as in figure 13.

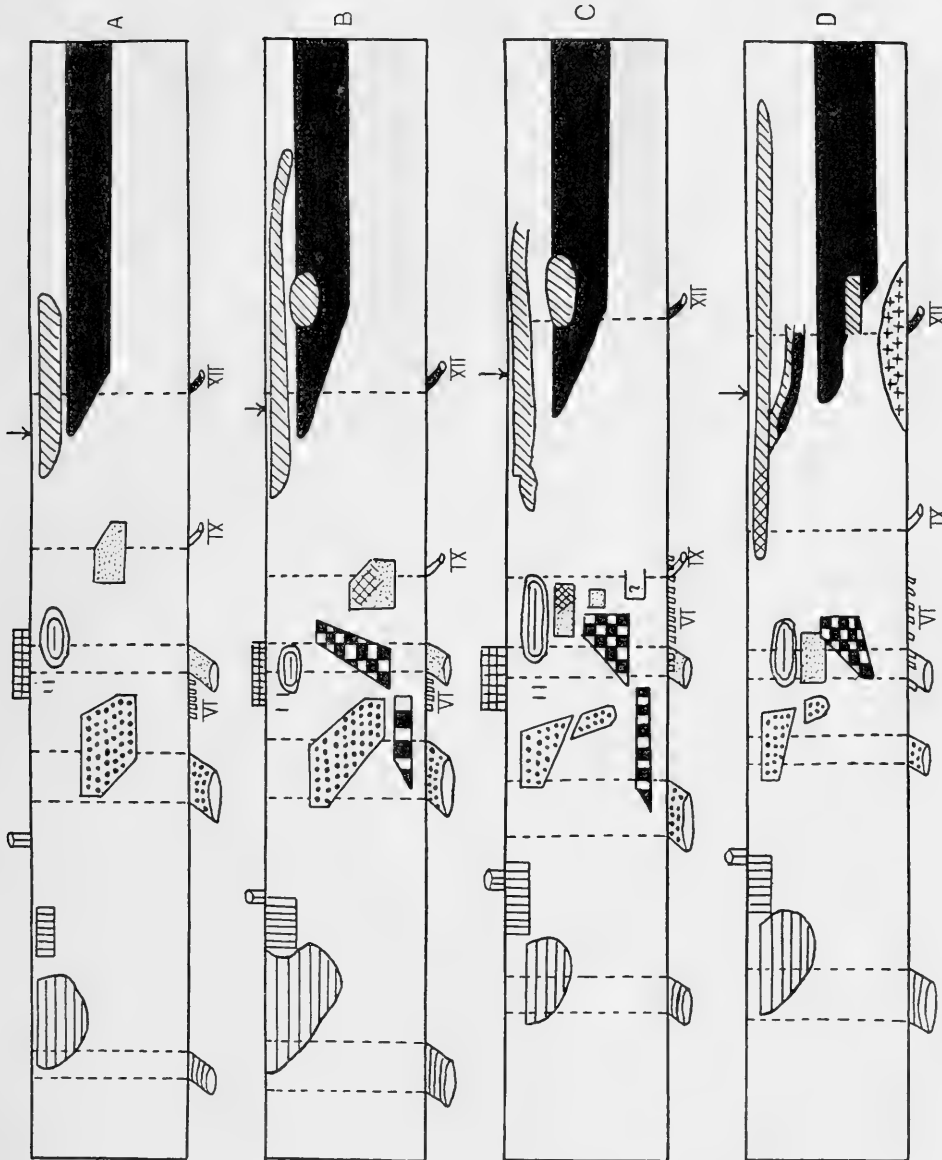


Fig. 15 Reconstruction charts of motor roots and nuclei after Kappers (34). A, *Boa constrictor*; B, *Varanus salvator*; C, *Alligator sclerops*; D, *Casuaris australis*. Signs and abbreviations as in figure 13.

DISCUSSION

1. *Intermedius complex*

Shortly after the publication of Brandis' researches (l.c.), Fürbringer (24, p. 504) commented on the probable existence of a correlation between the degree of differentiation of the dorsal XII nucleus in birds and that of their syringeal muscular apparatus. Kappers subsequently has pointed out on numerous occasions that the central association of vagal and hypoglossal elements within the avian nucleus intermedius was highly significant in view of the fact that the musculature of the syrinx peculiar to birds is innervated by XII fibers, while that of the simple larynx in these forms derives its supply from the vagus nerve. The evidence collected by this author (see especially 34), while not conclusive, is strongly in favor of considering the nucleus intermedius X as the motor laryngeal center, while doubt can no longer remain as to the innervation of the syrinx musculature largely if not exclusively from hypoglossal neurones of the intermedius complex.

In the simply organized laryngeal apparatus in birds but two intrinsic muscles, an apertor and a sphincter, are present, while in the syrinx one or more pairs of proper vocal syringeal muscles, in addition to the paired extrinsic m. tracheo-clavicularis or m. sterno-trachealis, are present in the vast majority of birds. These facts for the most part accord well with the observations noted above that in the majority of birds examined a definite X-XII intermedius complex is present.

There are, however, certain birds in which the syrinx is devoid of intrinsic musculature. All members of the family Ciconiidae lack true vocal syringeal muscles. They are absent also in a few members of the group Gallinae, as well as in *Struthio*, *Casuaris*, and a number of other forms. It is probable that in these birds the absence of intrinsic syringeal musculature is to be considered due to the loss of muscular elements originally represented rather than to the survival of a primitive character (cf. Newton and Gadow, 44; Beddard, 4). Such a conclusion would receive support from the fact that in both *Ciconia* and in

Casuaris (34) a quite well-marked X-XII intermedius complex has been observed, while in *Struthio* according to Brandis (13, pp. 630 and 644), though both vagal and hypoglossal parts of the intermedius complex are small, yet there is no doubt as to their presence.

There remains to be considered in this connection those forms in which the nucleus intermedius appears to be largely (if not entirely) composed of elements of but one type, either vagus or hypoglossal, and which fall within groups I or III as defined above on pages 238 and 242.

Unfortunately, the motor nuclei and roots in none of the birds constituting group I have been charted, and the only available information on the central relations of the motor X and XII roots and nuclei is that furnished in Brandis' description (l.c.). The group in question constitutes a quite heterogeneous collection of seven forms belonging to no less than five different orders (Evans' classification). Further, with the exception of *Phasianus* and *Numida*, on the plan of whose syringeal organization I have been unable to obtain details, each of the remaining five members of the group is equipped with a well-developed pair of intrinsic syringeal muscles. In this respect, therefore, these birds are as specialized as many of the forms included in group II.

It may appear that an origin of XII roots largely if not entirely from the slightly differentiated rostral part of the cervical motor column might well be a primitive character in birds. On the other hand, the very heterogeneity of the group in which this character has been described, as well as the close relationship evidently existing between its members and those of group II, argues strongly against the fundamental importance of this feature. It may be concluded, therefore, that, even should further investigations confirm Brandis' observations, the absence of a hypoglossal component in the nucleus intermedius complex of these forms should be considered rather as a specialization away from the type characteristic of group II than as the retention of a primitive character.

The few forms comprising group III, however, all belong to a small quite sharply defined and specialized natural group.

In *Cacatua roseicapilla* the rostral extremity of the nucleus intermedius XII is practically on a level with that of the dorsal motor vagus nucleus, a relation similar to that obtaining between the motor vagus and hypoglossal nuclei in many mammals. Unlike the latter forms, the caudal extremity of the nucleus intermedius XII in *C. roseicapilla* extends relatively far below that of the dorsal vagus column. *Cacatua* differs from the other birds examined in these respects, as well as in the greater relative length of its nucleus intermedius XII and in the exceptionally rostral level at which the first hypoglossal rootlet makes its exit (cf. figs. 15 D and 16).

So far as can be gathered from Brandis' descriptions and figures, the relations of the nucleus intermedius XII in *C. galatea*, *Melopsitticus*, and *Palaeornis* are very similar to those in *C. roseicapilla*. It would seem, therefore, that in parrots the specialization of a central hypoglossal intermedius cell group has progressed under influences which differ in some important fashion from those operating to produce what may be termed the typical avian intermedius cell complex.

It has already been noted that the cell group here termed the typical avian intermedius complex has probably been evolved largely as the central expression of a peripheral specialization in the sound-producing apparatus peculiar to birds wherein a specialized somatic syringeal musculature works synergically and coordinately with a simple visceral laryngeal musculature. If this be so, it will be of interest to inquire if any peripheral specializations of the syrinx or other organ obtain among parrots which might account for the presence in these forms of an intermedius XII nuclear development unique among birds.¹⁰

¹⁰ Bath (2 and 3) has shown that taste buds do not occur on the horny tongue epithelium of birds, though they are found in small numbers in the mouth region in the majority of these forms, chiefly near the entrance to the pharynx and glottis and in the mucosa of the buccal floor and margins of the jaws and to a lesser extent on the palate. In mammals Kappers (9) has pointed out that the distribution of taste buds innervated by VII and IX fibers over the surface of the tongue is to be correlated with the rostral and dorsal position of the hypoglossal nucleus close to the taste center of these forms. In parrots, however, though the total number of taste buds (i.e., 300 to 400) is much greater than in any other avian form, it is evident, in view of their location elsewhere than in the lingual mucosa, that incoming impulses from this source can play no such rôle in determining the location of the XII nucleus.

The intrinsic muscles of the syrinx among parrots, though well developed, are not so numerous (but three pairs) nor so highly differentiated as they are, for example, in *Corvus* (49) and in most Oscine birds (4), where either five or seven pairs of syringeal muscles are usually present.¹¹

On the other hand, Mudge (42), Kallus (*Melopsittacus*, 31), and others have shown that in contrast to all other birds the chief bulk of the tongue in parrots consists of its highly differentiated intrinsic musculature which extends as well in the anterior third as in the caudal portions of this organ.

The possibility of coordinate lingual action during phonation in parrots was noted long ago by Owen (47, p. 225). It would seem probable that this surmise is correct (Denker, 20) and that the tongue in parrots forms an integral part of the sound producing mechanism in these animals, and by alteration of its shape may be capable of modifying in no unimportant manner the quality of the tones produced by syringeal vibrations.

The large size and unique specialization of the nucleus intermedius XII in parrots is thus evidently to be correlated with the bulk of their lingual musculature and especially with the complexity of its arrangement and action, and not with any syringeal peculiarities in these forms.

In the development of its intrinsic musculature and in the part it may play in phonation as well as in deglutition the action of the tongue in parrots in some respects resembles that of this organ in many mammals. It is, of interest, therefore, to note the close association of the nucleus intermedius XII with the dorsal part of the caudal visceral motor column in parrots in view of the analogous motor nuclear association in this region in mammals.

2. *Visceral motor nuclei*

Nervus accessorius. In all lower forms so far investigated the accessorius nucleus when present is evidently but the caudal prolongation of the dorsal motor vagus column (8, 9, and 10).

¹¹ An excellent short summary of the number and arrangement of syringeal muscles in various groups of birds is given by Newton and Gadow (44, p. 939). See also Weiss (61, p. 297) and Beddard (4).

According to Brandis' observations (13, p. 634-5), the avian accessory nucleus becomes continuous at its rostral end with the cell group which has been described above as the nucleus intermedius. It would appear also that Turner's observations (50) confirm Brandis' description, since the latter author's 'nucleus dorsalis XII' evidently corresponds to Turner's 'nucleus of the spinal accessory.' The difference between the descriptions of Brandis and Lubosch (41) would also largely disappear if due allowance be made for the latter's failure to recognize the mixed X-XII character of the intermedius cell group.

Though some variation, no doubt, obtains in the rostral relations of the nucleus accessorius in birds, it is improbable in view of Bok's recent findings in *Gallus* (11) that this nucleus is in continuity with the dorsal motor vagus column in any adult avian form. In his careful ontogenetic study of the roots and nuclei of the vago-accessorius complex in the chick (i.e., pp. 511-512) the latter author has shown that the elements of the ventro-lateral vagus nucleus, together with those of the nucleus accessorius, originate from the dorsal motor vagus column and migrate ventro-laterad as one cell complex. Differentiation of these two nuclei becomes effected secondarily by the caudal migration of accessorius neurones. Thus, though the nucleus accessorius in both birds and mammals is primarily derived from the dorsal motor vagus column, yet it would appear that in birds its cells migrate first ventrad and later caudad to reach their final situation in the cord, while in mammals the reverse is true.

Nerves IX and X. It has been noted above that no vagus component was identified in the intermedius nucleus of *Cacatua roseicapilla*, though in *C. galatea*, *Palacornis*, and *Melopsittacus*, Brandis (i.e.) was able to demonstrate its presence. However, in view of the great similarity in the peripheral, lingual, laryngeal, and syringeal equipment of *C. roseicapilla* and *C. galatea*, it is probable that my failure to identify the component has not been due to its entire absence, but rather to the very extensive development of the hypoglossal elements of this complex.

It is well known that both the crop and gizzard musculature of birds are under vagus control (Biedermann, 5, p. 1206). Vermeulen (55) has shown that in many forms a definite correspondence obtains between the size of the dorsal motor X nucleus and the development of the stomach, and similar observations had earlier been made by Kosaka and Yagita (38) in the case of birds. In all parrots a crop is present and, though in cockatoos this structure is of no great size, yet it is well developed. A small gizzard is also present in these animals (v. Oppel, 45, 46; Owen, 47, p. 161 et seq.), so that the specialization and relative size of the stomach in *Cacatua* would compare favorably with the development of this organ in *Spheniscus* and *Colymbus* (23 and 60). It thus becomes difficult to account for the short and relatively slightly developed dorsal motor vagus nucleus in *Cacatua* which in the proportions of this cell column differs so markedly from the other birds examined.

The ventro-lateral motor X nucleus of birds in some respects resembles that of *Varanus* and *Alligator* (cf. fig. 15), but is more rostrally placed than in the latter forms. The possible significance of this condition will be discussed subsequently.

It is interesting to note that the motor IX nucleus in all birds examined is characteristically associated with the dorsal motor vagus cell group and forms thereby the most rostral part of the caudal visceral motor column. In discussing the relations of the motor nuclei of this region in reptiles (10), it was pointed out that in these forms the association of glossopharyngeal with facial motor perikaryons rather than with vagal elements would seem to be due to a rearrangement of the visceral motor nuclear pattern largely as a consequence of the loss of the hyobranchial pump mechanism for pulmonary ventilation and, as Kappers has noted, under the direct influence of the caudal VII-IX taste center.

In birds, also, the motor glossopharyngeal and vagus nerves, apart from the control of the simple laryngeal muscles, have lost entirely their original function of innervating respiratory musculature, the effectors concerned in pulmonary ventilation in these forms being wholly of striate somatic character.

The gustatory organs of birds are but poorly developed (vide supra) and the pars intermedius VII in these forms is reduced to a minimum. Kappers (36) has shown that but few gustatory fibers can enter the brain stem by the latter path in these forms, the majority of the special visceral afferent impulses being transmitted by the glossopharyngeal and vagus nerves. This author pointed out further that the gustatory IX-X components terminate in a nucleus which is evidently the homologue of the mammalian dorso-lateral and dorso-median nuclei of Staderini.

The IX-X pharyngeal and oesophageal musculature in birds acts coordinately during deglutition or in antiperistaltic movements of the foregut in practical independence of V-VII effectors. This reflex action of the dorsal motor IX-X nucleus is chiefly inaugurated by afferent impulses of both general and special visceral nature which enter the brain stem almost wholly through the sensory roots of the two nerves in question. The close association of the dorsal motor IX and X nuclei in the immediate neighborhood of the terminal special visceral sensory nucleus of their own roots is thus another illustration of Kappers' principle of neurobiotaxis.¹²

Motor V-VII complex. In Kappers' earlier publications (see especially 7 and 12) special attention has been drawn to the remarkable fact that in most birds, in contrast to all other vertebrates, the motor VII nuclei (two or three in number) are situated wholly rostrad of the exit level of their motor root and in close association with the motor V cell groups. An exception to this rule among birds has been encountered only in the case of *Casuaris*, where the motor VII nucleus lies on the exit level of its motor root. Kappers has further shown that the position of the motor VII nucleus of birds rostrad of its root exit and its close association with the motor V nucleus is largely due to a dominating trigeminal reflex influence in the absence of a well-developed gustatory center.¹³

¹² A similar association of IX-X motor nuclei due to analogous circumstances has already been observed in Cyclostomes (fig. 13 A, p. 257).

¹³ For a full discussion of this interesting question reference should be made to Kappers' original communications (l.c.).

Kosaka and Hiraiwa (39) have identified with considerable accuracy the muscular localization within the VII motor nuclei in *Gallus* and *Anas*. The 'Hauptkern' of these authors was found to be chiefly concerned with the innervation of the m. subcutaneous colli; the 'Nebenkern' with the m. mylohyoideus posterior (m. hyomandibularis and lateralis of Futamura, 25), and the 'Digastricus-kern' with the m. digastricus (m. depressor mandibulae of Adams, 1). With these facts in mind, it will be of interest to consider further the V-VII nuclear pattern in *Ciconia* and *Cacatua* (fig. 16).

In *Ciconia* the 'Nebenkern' is not represented as a separate cell group in correspondence with the fact that the tongue is small and not protractile.¹⁴ In this form the elements are probably incorporated within the dorsal motor VII nucleus which represents the 'Digastricus-kern' of Kosaka and Yagita. The ventral motor VII nucleus in *Ciconia* is larger than that in *Cacatua* in correspondence with the more extensive development of the m. subcutaneous colli in the former animal.

In *Cacatua* an intimate association of VII 'Digastricus-kern' and motor V elements occurs in what has been termed in the foregoing description the combined V-VII motor nucleus. The VII motor elements of the complex are more numerous than the V and the 'Nebenkern' of Kosaka and Yagita is probably represented here by the caudo-ventral prolongation of this nucleus described above.

The full action of the m. pterygoideus anterior in elevating the maxilla in birds is not possible except when the mandible is widely opened by reason of the contraction of the m. depressor mandibulae (Biedermann, 6). Adams (1) has drawn attention again to the importance of this action of the m. depressor mandibulae (m. digastricus) in the Psittaci.¹⁵ In view of these facts,

¹⁴ When the tongue is very protractile or very thick, the m. mylohyoideus posterior consists of two parts termed by Gadow the m. serpi-hyoideus and m. stylo-hyoideus, both innervated by the facial nerve (Newton and Gadow, 44; Biedermann, 7).

¹⁵ The so-called m. digastricus of birds which is wholly innervated by the facial nerve is better termed the m. depressor mandibulae, since it is not the homologue of the mammalian muscle of the same name whose anterior belly is innervated by the trigeminal nerve (Adams, 1).

the close association of certain trigeminal and facial elements within the limits of a common V-VII nucleus is highly significant as an indication of the probability that these motor V cells represent elements governing the action of the m. pterygoideus anterior.

3. *Eye-muscle nerves*

Nerve VI. But little remains to be said concerning the abducens nerve, which in most respects differs but little from that of reptiles. An interesting series of gradations in the exit level of its motor rootlets is shown in figures 15 and 16. In *Cacatua*, *Ciconia*, and *Colymbus* these emerge rostrad of the exit level of the motor VII root; in *Spheniscus* their emergence is both rostrad and caudad of this level, but in *Casuaris* the primitive condition is for the most part retained and most of the abducens rootlets in this form emerge caudad of the exit level of the motor root. The dorsally placed abducens nucleus is located for the most part on, or slightly rostrad of, the motor VII root exit, and is somewhat larger than the reptilian abducens cell group, a fact which, as Kappers pointed out, is to be correlated with the greater complexity of the musculature which it supplies in birds (32, 34).

Nerves III and IV. In all birds examined the trochlear nucleus is in close apposition with and frequently overlapped by the caudal end of the oculomotor cell group. The apposition of these two nuclei is evidently due to the rostral migration of the former, whose cells have been shown by Bok (11) to have their ontogenetic origin a considerable distance caudad of the oculomotor cell group. Though the position of both oculomotor and trochlear nuclei in relation to the exit levels of the oculomotor and motor V roots is subject to considerable variation, the exit level of the trochlear root in relation to its nucleus is relatively constant. In *Casuaris*, *Cacatua*, and *Ciconia* it emerges at the caudal end of the trochlear nucleus in *Colymbus* at the midlevel of the nucleus, and in *Spheniscus* at its rostral end (cf. Kappers, 34). Thus in no case among birds does the trochlear decussation and emergence take place below the level of its own nucleus as is commonly observed in lower forms (cf. fig. 14).

The development of subsidiary cell groups within the oculomotor nucleus has attained to a high state of complexity among birds in correlation with the perfection of the intrinsic and extrinsic oculomotor effectors. In the descriptive portion of this paper special attention was drawn to the accessorius cell group which in its relations, staining reactions, and in the morphology of its elements closely resembles the Edinger-Westphal nucleus of mammals with which it had been homologized by Brandis. In birds there can be no doubt that certain oculomotor root fibers arise in this cell group, and for this reason, especially, Kappers' term nucleus accessorius III has been retained (*vide supra*, footnote 9, p. 250).

Brouwer (17) has recently reinvestigated the Edinger-Westphal nucleus both from the clinical and from the comparative anatomical and pathological standpoint, and he has concluded that there exists the strongest evidence confirming Jacobsohn's view that the Edinger-Westphal nucleus is the nucleus sympatheticus nervi oculomotorii (30).

In a consideration of the homology of the nucleus accessorius III of birds Kappers' earlier description of a well-developed nucleus accessorius III in one specimen of *Varanus* sp.? becomes significant, since it is thereby shown that an oculomotor accessory cell group (probably of sympathetic nature) has been evolved among reptiles and is present in certain modern representatives of these forms in a position exactly analogous to that of the Edinger-Westphal nucleus of mammals and the nucleus accessorius III of birds.

CONCLUSION

The cerebral motor nuclear pattern in birds, while showing some important variations in different avian families, is on the whole fundamentally similar in all the forms examined, and characteristically different from that obtaining in any other vertebrate group. Thus the strikingly specialized nature of the modern class Aves is well exemplified also in the topographical relations of the motor roots and nuclei of their cerebral nerves.

One of the most characteristic features of this avian nuclear pattern lies in the association of the V-VII motor nuclei and the situation of the facial motor nuclei on, or more frequently rostral to the exit level of their motor root. It has been pointed out above that a similar association of V-VII motor nuclei is not found as a group character elsewhere in the vertebrate phylum except in cyclostomes. The association of facial and trigeminal motor nuclei in birds would seem to be due largely to the dominant influence of sensory trigeminal impulses upon the reflex action of both facial and trigeminal musculature in those forms. The mutual association of the V-VII motor nuclei in close contiguity with the chief sensory center acting reflexly upon them thus affords a striking illustration of Kappers' neurobiotactic concept.

An association of the motor glossopharyngeal and dorsal motor vagal nuclei, such as characterizes birds, is encountered as a group feature elsewhere among vertebrates only in petromyzonts.¹⁶ In birds, though the IX-X musculature has completely lost its primitive respiratory function, yet the effectors concerned act synergically and coordinately in all movements of the foregut and are dominated reflexly by visceral sensory impulses entering by way of the afferent IX and X roots. Thus these motor nuclei also are associated in the neighborhood of the chief center acting reflexly upon them.

The intermedius X-XII motor complex constitutes a third characteristic feature of the nuclear pattern within the brain stem of birds, and one apparently unique among vertebrates. It has been shown to be highly probable that this peculiar complex was evolved as a consequence of the development of the laryngo-syringeal mechanism peculiar to birds. Its possession by certain forms, such, for example, as *Struthio*, *Ciconia*, and *Casuaris*, in which intrinsic syringeal musculature is lacking, constitutes strong evidence in favor of considering the relatively simple syringeal organization of these animals to be due to a

¹⁶ Among teleosts, although a similar nuclear association has been observed sporadically in various forms (e.g., *Ameiurus*, 8), in no case does this character constitute a group feature.

reduction in the number of elements originally present and not to the retention of a primitive character.

In the great development of the hypoglossal component of their intermedius motor complex, it has been shown that parrots differ from all other birds. This fact would appear to be correlated with the exceptional development and differentiation of the intrinsic tongue musculature among members of the parrot family. In its morphology and relations the hypoglossal nucleus in parrots resembles in many respects that of mammals, though the peculiar psittacine nucleus cannot well have been evolved as other than a specialization of what has already been termed a typical avian intermedius complex (*vide supra*).

Intrinsic oculomotor nuclear differentiation has attained to a high state of complexity among birds, and the ground-plan of this avian oculomotor specialization is essentially similar to that obtaining in this nucleus in many mammals. In this connection it is significant that Kappers has described an arrangement of the intrinsic oculomotor nuclei in one specimen of *Varanus* sp.? which closely resembles that obtaining in birds (*vide supra*, pp. 255 and 270). The occurrence of this phenomenon in a modern reptile shows definitely that the ground-plan of the nuclear differentiation characteristic of this region in higher forms has already been determined within the class from whose prototypes both avian and mammalian forms were evolved.

Finally, it would appear that modern birds and reptiles while presenting minor resemblances, show a fundamentally different plan of organization in the arrangement of their cerebral motor nuclei, though either avian or reptilian motor pattern could well have been evolved from a form whose nuclear organization was of a type similar to that obtaining in some modern anurans (e.g., *Rana catesbiana*, fig. 14 B)¹⁷ or urodeles (e.g., *Triton*, fig. 14 A).

¹⁷ It has previously been pointed out (9, p. 423) that though the anuran type has certainly been evolved comparatively recently in vertebrate phylogeny, yet "within the brain stem in *Rana* a motor nuclear pattern obtains which on first examination would seem to be much more primitive than the motor nuclear pattern in selachians."

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Resumen por el autor, H. Saxton Burr.

El desarrollo temprano de los hemisferios cerebrales de
Amblystoma.

El estudio experimental del destino del neuroporo de *Amblystoma* y un nuevo exámen de los estados tempranos de la formación de los hemisferios cerebrales de dicho animal han demostrado los siguientes hechos: El labio ventral del neuroporo, después de haberse reunido los labios laterales, se transforma en la cresta terminal del embrión, la cual es invadida más tarde por la comisura anterior. La fusión de los labios laterales del neuroporo forma la lámina terminal, la cual, por consiguiente, termina en el borde anterior de la cresta terminal. La placa del piso de His no se extiende anteriormente a la fovea del istmo. El intervalo en la línea media ventral del tubo neural está ocupado de delante atrás por la continuación de un lado a otro de la placa alar anteriormente, y por la de la placa basilar posteriormente. Como resultado de esto el material evaginado para formar el hemisferio cerebral se deriva enteramente de la placa alar, y a partir de aquí la laminación dorso-ventral se impone secundariamente sobre la disposición de las partes en dirección céfalo-caudal.

Translation by José F. Nonidez
Cornell Medical College, New York

THE EARLY DEVELOPMENT OF THE CEREBRAL HEMISPHERES IN AMBLYSTOMA

H. SAXTON BURR

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TWENTY-SIX FIGURES

Since the researches of His ('88, '92, '93) his original concept of the structure of the neural tube has been very generally accepted. The work of subsequent investigators well known in the literature has established the main principles of the work of His beyond a reasonable doubt. In his early papers, however, he did not elaborate very fully the rostral relations of the longitudinal zones of the neural tube. It was his belief that these four primary columns meet at the ventral lip of the neuropore. On the basis of this assumption, the roof and the floor plates separate the neural tube into two independent lateral halves. It is evident, therefore, that the fate of the ventral lip of the neuropore becomes a matter of prime importance. This matter has been studied carefully by workers, among whom may be mentioned Johnston ('05, '09) and Schulte and Tilney ('15). In all the work that has been done, however, it has been tacitly assumed, as originally pointed out by His, that the floor plate terminated at the ventral lip of the neuropore. The exceedingly interesting and suggestive paper of Kingsbury ('20) contains the first suggestion that this assumption is not altogether justified by the facts. During the progress of an investigation of the early development of the cerebral hemispheres in Amblystoma it became evident that a more careful study of the rostral relations of the longitudinal zones of His was necessary. Several years ago Doctor Herrick suggested that a more detailed study of the early development of the cerebral hemispheres might yield valuable data. The researches of Bindewald ('14), Crosby ('17), Heuser ('13), and others have shown in many forms the fundamental character of the telen-

cephalic evagination. In all the cases studied the outpouching of the wall of the neural tube has been closely associated with the olfactory placode. Beginning first in the cyclostomes as an evagination of the olfactory bulb, the further phylogenetic history has involved progressively more and more olfactory association material (Herrick, '21). The relation of the evaginated portions to the rest of the tube assumes, therefore, a prime importance. The following communication deals with an experimental study of the fate of the neuropore in *Amblystoma* and the subsequent history of the relations of the longitudinal columns of the neural tube in this region.

THE FATE OF THE NEUROPORE

The work of Johnston ('09), based on a comparative study of the forebrain vesicle in vertebrates, showed clearly that the ventral lip of the neuropore was incorporated into the brain as the terminal ridge lying between the lamina terminalis anteriorly and the chiasmatic ridge posteriorly through the fusion of the lateral lips of the blastopore. He concluded that the preoptic recess, marking the termination of the sulcus limitans and separating the terminal ridge from the chiasmatic ridge, represented the meeting-point of the roof and floor plate and hence the anterior end of the neural tube. Hatschek ('09) confirmed in the main Johnston's researches, but stated that the terminal ridge or 'Basilarlippe' marked the primary anterior wall of the neural canal. In any event, it seems evident that this terminal ridge is subsequently occupied by decussating fibers of the anterior commissure.

To determine experimentally the above facts, a number of operations were performed on *Amblystoma* larvae. At the suggestion of Doctor Harrison, fine hairs were inserted into the neuropore for a short distance and watched during subsequent development in the hope that the ultimate position of the hair would indicate the final position of the neuropore in the embryo. Difficulties were at once encountered, since a fine hair, unless inserted for a considerable distance into the embryo, would not remain there, but would be extruded. If, on the other hand, the

fine hair was inserted far enough to prevent its extrusion it became evident that the imbedding of the hair in the tissue deep in the embryo prevented its following the ventral path of the neuropore. However, by making a slight wound in the ventral lip of the neuropore and staining it with Nile-blue sulphate, according to the method of Detwiler ('17), it was found that the stained area could be followed throughout the subsequent development. In fact, the Nile-blue sulphate remained in the tissue for a period

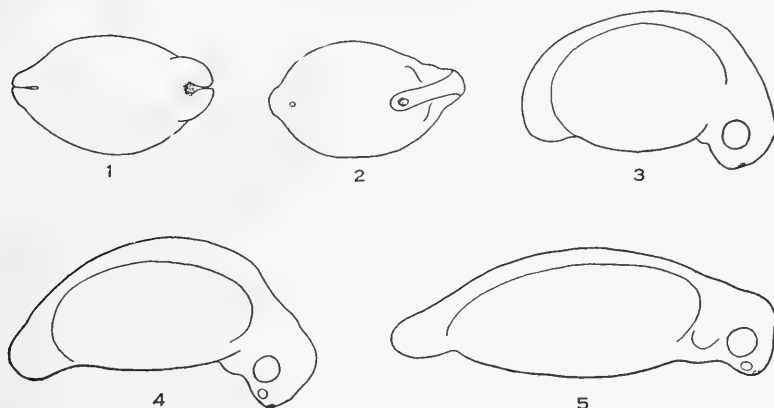


Fig. 1 Ventral view of *Amblystoma* embryo, showing area of ventral lip of neuropore stained with Nile-blue sulphate, stippled. $\times 12$.

Fig. 2 Same embryo 24 hours later. $\times 12$.

Fig. 3 Lateral view of same embryo 48 hours after operation. $\times 12$.

Fig. 4 Lateral view of same embryo 72 hours after operation. $\times 12$.

Fig. 5 Lateral view of same embryo 96 hours after operation. $\times 12$.

of twenty-one days. A series of drawings showing the position of this stained area is given in figures 1 to 5, representing an elapse of four days. A sagittal graphic reconstruction of the brain of the embryo shown in figure 5 is given in figure 6. The region of the stained area is indicated in the figure, *rn*.

The material which makes up the ventral lip of the neuropore produces the ridge in the floor of the neural tube to which Johnston has given the name of the terminal ridge (fig. 6, *tr.*). It is evident, therefore, that the closure of the lateral lips of the neuropore produces the lamina terminalis which ends not in the pre-

optic recess, as Johnston indicated, but in the terminal ridge. By this fact it may be seen that the terminal ridge marks the rostral limit of the roof plate of His.

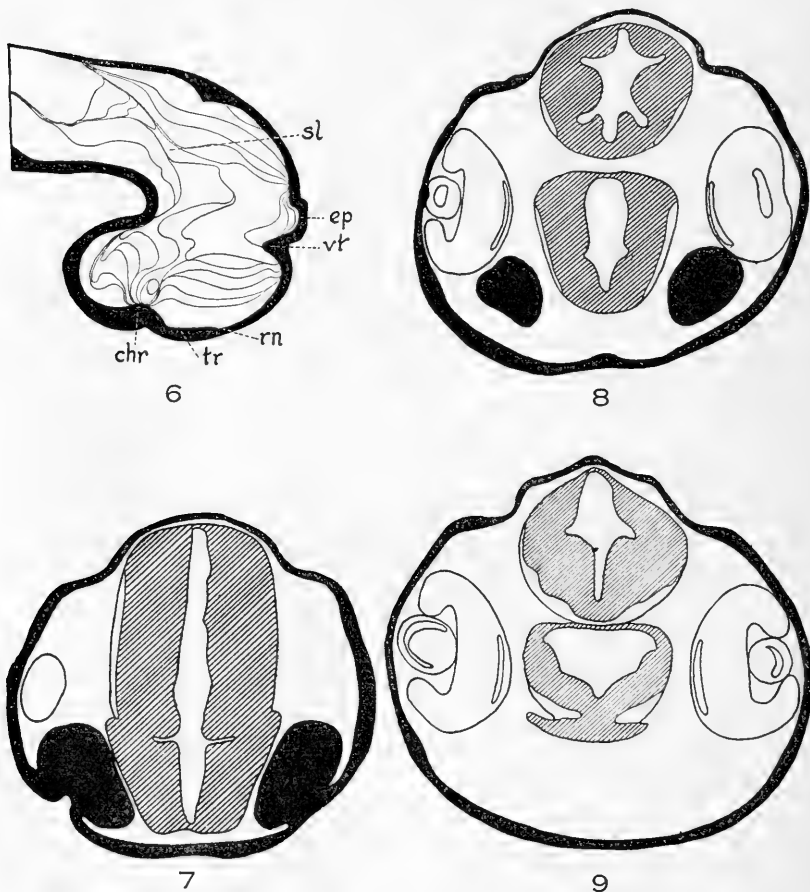


Fig. 6 Graphic reconstruction from sagittal sections of brain of embryo shown in figure 5. $\times 50$. *chr.*, chiasmatic ridge; *ep.*, epiphysis; *rn.*, neuropore; *sl.*, sulcus limitans; *tr.*, terminal ridge; *vt.*, velum transversum.

Fig. 7 Transverse section through the head of an 11-mm. *Amblystoma* embryo, showing the general relations of the lamina terminalis. $\times 50$.

Fig. 8 Transverse section 190 μ caudal of figure 7, showing the general relations of the median alar plate. $\times 50$.

Fig. 9 Transverse section 110 μ caudal of figure 8 passing through the chiasmatic ridge. $\times 50$.

Now the ventral lip of the neuropore is the region at the rostral end of the neural tube where the material which makes up the lateral lips of the neuropore is continuous from side to side across the midline. This material constitutes the rostral portion of the alar plate, and hence we may conclude that in the terminal ridge the alar plate of one side becomes continuous with that of the other. In other words, the assumption of Kingsbury ('20) that the alar plate of the neural tube forms an arch about the anterior end of the neural plate may be considered as correct. The terminal ridge represents the median portion of the alar plate with its caudal boundary marked by the preoptic recess where the sulcus limitans of one side becomes continuous with that of the opposite side.

The further suggestion of Kingsbury that the basal plate is likewise continuous from side to side across the median line arching around the fovea isthmi, the anterior limit of the floor plate of His, seems to be substantiated by the following facts. In figures 7 to 12 are shown a series of critical cross-sections through the brain of *Amblystoma* showing the relations and structures, respectively, of the lamina terminalis, the terminal ridge, and the chiasmatic ridge. The gross relationships are shown in figures 7, 8, and 9 and the microscopical structure in figures 10, 11, and 12. It is evident from these figures that from the point where the lamina terminalis merges with the terminal ridge the midventral line of the brain is occupied, not by typical floor-plate material, i.e., non-nervous supporting tissue, but throughout its length until the fovea isthmi is reached, by typical nervous tissue. Only one exception can be made to this general statement and that is that part of the floor plate which is to form eventually the roof of the hypothalamus. In the older stages it is reduced to a non-nervous lamina. This, however, has no significance when it is noted that this region in its early developmental stages does not differ from the structure of the terminal ridge and the chiasmatic ridge, so far as can be determined from microscopic sections. The thinning of this area is shown in figures 14, 16, and 18.

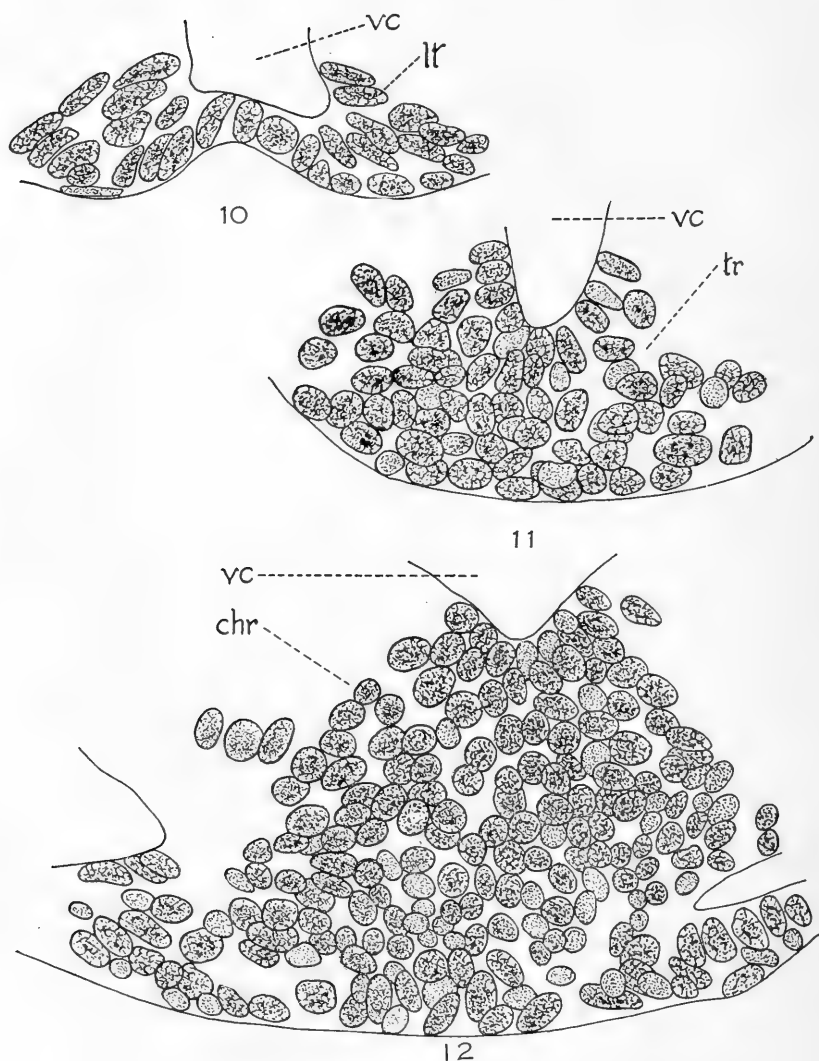


Fig. 10 Section through lamina terminalis, showing cellular detail. $\times 175$. *lt.*, lamina terminalis; *vc.*, ventricular cavity.

Fig. 11 Section through median alar plate, showing arrangement of cells. $\times 175$. *tr.*, terminal ridge; *vc.*, ventricular cavity.

Fig. 12 Section through chiasmatic ridge, showing the continued cellular increase in the median basal region. $\times 175$. *chr.*, chiasmatic ridge; *vc.*, ventricular cavity.

It would seem, therefore, that from the terminal ridge caudally to the fovea isthmi we are dealing not with the non-nervous floor plate of His, but with a nervous tissue continuing the basal plate of one side with that of the other. So far as *Amblystoma* is concerned, it is probable that the fundamental arrangements of the longitudinal columns of His in the rostral end of the neural tube were as indicated by Kingsbury ('20) in his figure 6. These rostral relations may be stated as follows: The floor plate of His terminates at the fovea isthmi, about which arches anteriorly the basal lamina, the continuity of which is maintained by the ventral portion of the neural plate lying between the fovea and preoptic recess. Arching about the basal lamina still more rostrally and separated from it by the sulcus limitans, is the alar lamina. The continuity of the two lateral alar laminae is represented in the midline by the terminal ridge. The roof plate whose rostral portion is the lamina terminalis, therefore, does not meet the floor plate, but is separated from it by the structures above mentioned.

EVAGINATION OF HEMISPHERES

The general form and arrangement of the constituent elements of the forebrain of *Amblystoma* are well known through the researches of Herrick, Johnston, and others. As a result of their work, and more particularly that of Herrick ('10, '21 a), we know that the forebrain of *Amblystoma* has a fairly well-defined organization. Through his study of *Amblystoma* larvae of varying stages and of the adult, Herrick has made fairly definite the following parts of the hemisphere. It can be divided into a number of relatively distinct nuclei. The most important of these is the olfactory bulb lying at the rostral end of the hemisphere. In addition, four regions are described called, respectively, the ventro-medial, ventro-lateral, dorso-medial, and dorso-lateral parts. These converge forward into a relatively undifferentiated nucleus olfactorius anterior at the base of the olfactory bulb.

The ventro-medial part is the septum (in the broad sense), characterized by the ventro-medial olfactory tract, the medial forebrain bundle and their connecting tracts. The ventro-lat-

eral part in *Amblystoma* is an undifferentiated strio-amygdaloid body, characterized by the ventro-lateral olfactory tract, the lateral forebrain bundle and their connecting tracts. The dorso-medial part is the primordium hippocampi, receiving a small dorso-medial olfactory tract, but chiefly characterized by associational fibers relating it with contiguous parts of the hemisphere and by strong tracts directly to the epithalamus and hypothalamus. The dorso-lateral part is the relatively undifferentiated precursor of the lateral olfactory nucleus and pyriform lobe of higher brains, receiving (among others) the strong dorso-lateral olfactory tract, terminals of the lateral forebrain bundle, and associational fibers from the dorso-medial part.

The analysis of the early phases in the development of these structures has been carried out on a series of larvae of *Amblystoma punctatum*. Wax-plate models of the brains of five larval *Amblystoma* were made and checked by microscopic dissection of the entire brain. Drawings of three of these are shown in figures 13 to 18. In addition careful study was made of transverse sections stained in haematoxylin and erythrosin to determine the microscopic relations. A few of the critical sections are shown in figures 19 to 26.

It was noted in a previous paper that in general the form of the regenerating hemisphere followed the same pathway of development as the normal forebrain. Further analysis has shown that the detailed genesis pursues a similar course. This suggests a rather interesting problem. It has been shown that a regenerating hemisphere is derived from a thin layer of typical ependyma cells which bridge over the interventricular foramen after removal of the hemisphere (Burr, '16 b). These ependyma cells are derived from the ependymal cells surrounding the interventricular foramen. These are then new cells derived from cells not specifically involved in the cerebral evagination, and yet these new cells are capable of developing a new hemisphere which repeats in its genesis the normal development of the forebrain. The question at once arises, is the pathway over which this development must go determined in the ependymal cells themselves or is it laid down by external factors dependent on the relation

of the cells to the surrounding tissues? The probability is that neither of these factors operates to the exclusion of the other. For it has been shown that when the mass of nerve cells which is to form a cerebral hemisphere is transplanted to some other part of the larva, the general arrangement of the parts is maintained in the subsequent development (Burr, '20). This would indicate that the inherent factor in the development of these ependyma cells was strong. But we have in the regenerating brain a new hemisphere formed from cells which, since they do not normally form a hemisphere, nevertheless acquire the inherent factors which determine the path or course of development. A description of the critical stages in this pathway of development follows.

The medial aspect of the lateral wall of the neural tube of a stage 30 (Harrison) shows the rather strongly marked sulcus interencephalicus anterior curving from a point, just cephalic to the chiasmatic ridge caudally, to the lamina terminalis cephalically. The anterior third of this sulcus is more deeply marked than is the posterior two-thirds and represents the site of the future evagination of the hemisphere (fig. 14). In somewhat earlier stages the cephalic boundary of the neural tube sweeps in an almost unbroken curve from the mesencephalic region above to the hypothalamic region below. But in the stage-30 brain a sharp indentation is evident immediately above the cephalic termination of the s. interencephalicus. It is produced apparently through the more rapid growth of the portions of the brain just above and below. By this process there is formed internally a crescentic ridge reaching horizontally from side to side of the neural tube, the velum transversum. Externally the indentation is carried caudally for a short distance to near the emerging optic tract, producing thereby the first signs of the ditelencephalic groove. It is bounded above by the prominent bulge of the thalamus.

At this stage the wall of the neural tube presents no clearly defined division into mantle and marginal layers, the radially arranged cells being scattered more or less uniformly through the wall (fig. 19). The wall of the tube in the region of the sulcus interencephalicus anterior shows a slight thickening and outward

bulging. That portion of the wall lying ventral to the sulcus shows an oval-shaped thickening which reduces the ventricle at this point to a narrow slit. This oval area represents the portion of the wall of the neural tube from which will develop the hemisphere. It lies immediately contiguous to the olfactory placode, although no connection between the two has been established at this time. As has been shown elsewhere (Burr, '16), this thickening is an inherited pattern of development, since it occurs regardless of the presence of the placode.

As soon as the ingrowing olfactory fibers reach the hemisphere there is evident the first external sign of evagination. The appearance of the neural tube at this point (stage 35, Harrison) is shown in figures 15, 16, and 20. Externally, the area of the lateral wall ventral to the bulging produced by the thalamus shows signs of its own outpouching. This outpouching is produced by the rapid proliferation of the cells lying in the neural wall parallel to the sulcus interencephalicus anterior from the velum transversum posteriorly to the lamina terminalis anteriorly.

The growth of the sheet of cells lying in this position swings the wall of the neural tube outward, deepening the external di-telencephalic groove and the s. interencephalicus internally. The latter from this point on is established as the sulcus angulus dorsalis of the hemisphere. There is still at this stage little change in the organization of the hemisphere wall, the radially arranged cells being scattered more or less evenly through it, though an area free of cells begins to appear at the surface of the neural tube.

From this point on a definitive hemisphere may be recognized. The di-telencephalic groove is deepened externally by the bulging of the dorsal margin of the hemisphere. This outward swing of the dorsal lip of the evagination first clearly defines the hemisphere. It bears no apparent relation to the nasal placode, since it occurs dorsally and anteriorly to it. The region of most rapid growth seems to lie between the posterior limit of the velum transversum, defined at the earlier stage, and the lamina terminalis. This area maintains its growth supremacy throughout

all the subsequent history of the hemispheres, since the lateral movement of the ventral area is reduced to a minimum.

This results necessarily in the major part of the evagination being directed slightly upward but mostly forward toward the anterior pole of the embryo (figs. 17 and 18). The internal configuration during this process has undergone a marked change. The median telencephalic ventricle is still but a narrow slit, while the forward growth of the hemisphere has developed the beginnings of the lateral telencephalic ventricle. The locus of the di-telencephalic groove is clearly seen through the presence of a cell-free area between the eminentia thalami and the dorsal part of the hemisphere (fig. 21). The ventral region of the evagination shows a slight lateral growth and the production of an interhemispheric groove in the mid-ventral line. The caudal boundary of the evagination is still rather indistinct, since little outpouching has occurred at this point. But the shallow sulcus limitans may be seen coursing past the posterior limit of the velum transversum to be lost in the preoptic recess. No markings on the medial wall of the hemisphere can be distinguished as yet, only the deep groove made by the anterior continuation of the sulcus interencephalicus anterior being evident. The internal structure of the hemisphere has undergone a marked change (fig. 21). The loosely packed arrangement of cells of the earlier stages has given place to a tightly organized neural wall. The rapid proliferation that has brought this about has resulted in a great increase in the number of cells, organizing the primitive arrangement into the ependymal, mantle and marginal layers. This elaboration is concomitant with the ingrowth of the olfactory nerve. While the presence of these fibers was undoubtedly the primitive stimulus producing the growth, the pattern of development has become so firmly fixed that the absence of olfactory fibers does not preclude differentiation. A slightly looser arrangement of cells in the lateral region presages the formation of the lateral forebrain tract. This tract appears as a thin band of peripherally arranged fibers in the pars ventro-lateralis of the hemisphere. The tract becomes more robust as the neural tube is followed caudally, indicating that at this time the majority of

its fibers are ascending to the hemisphere from the thalamus. No other defined group of fibers is evident at this stage. Only a very slight differentiation into the primary and secondary nuclei may be noted.

From this period forward development is but an elaboration of the processes so far described. At stage 40 the entire hemisphere is completely defined. The forward growth of the dorso-anterior pole of the hemisphere has progressed with the formation of the definitive lateral ventricles and septum endymale. The velum transversum is clearly defined joining the pars ventralis thalami of one side with that of the other. The lateral swing of the dorsal margin of the hemisphere has progressed and is accompanied by a slight increase in the evagination of the ventral margin.

The olfactory bulb may be seen clearly at this stage, occupying the ventro-lateral region of the anterior pole of the hemisphere. The ventro-median region of the pole is occupied by the nucleus olfactorius anterior. Running caudad from the bulb there may be distinguished in the ventro-lateral region of the hemisphere the tractus olfactorius ventro-lateralis incompletely separated from the lateral forebrain tract which lies immediately dorsal to it (fig. 23). The tract tends to disappear caudally in the region of the primitive corpus striatum which occupies the nuclear region of the hemisphere wall at the level of the posterior free margin of the velum transversum. Slightly dorsal to the lateral forebrain tract, the tractus olfactorius dorso-lateralis component appears as a thin lamina of fibers starting in the olfactory bulb and running caudad to disappear in the posterior pole. The pars dorso-medialis at this stage is a relatively thin layer of cells disappearing caudally into the ventral lamina of the velum transversum and thickening anteriorly as it becomes continuous over the anterior pole with the anterior olfactory nucleus. The septum endymale is entirely membranous except where it becomes continuous with the medial wall of the hemisphere at the level of the olfactory bulb. A very small group of fibers forming a ventral expansion of the white matter of the pars ventro-lateralis may be traced from the olfactory bulb as far caudally as the

ventral portion of the anterior commissure. This is the first evidence of the medial forebrain bundle.

From this point forward the pars lateralis of the hemisphere shows little change except in the increase in size, from the fundamental pattern already laid down (figs. 23 and 24). The pars dorso-medialis, however, shows at stage 42 (8 mm.) the first signs of differentiation into the primordium hippocampi. The single layer of ependymal cells lining the contiguous portion of the ventricle has, through proliferation, been converted into a rather thin, but nevertheless distinctly stratified layer (fig. 26). The ependymal cells constitute a clearly defined inner layer, peripheral to which lies a layer several cells thick more loosely packed than the inner layer. Among the outer loosely packed cells, in the caudal region may be seen the fibers of the fimbria complex forming dorsally a clearly defined lamina separating the telencephalon from the diencephalon. The stria medullaris complex cannot be distinguished at this stage.

The pars ventro-medialis shows the thickening anteriorly of the septum endymale. An increase in the number of mitotic figures in this region indicates an area of rapid proliferation. Concomitant with this growth, there is an invasion of the septum by fibers of the tractus olfactorius ventro-medialis. The enlargement thus formed constitutes the nucleus medialis septi. It seems doubtful, from the material at hand, that this nucleus develops as a result of the invasion of this region by cells from below and in front, as suggested by Herrick ('10). Rather, it would appear that the growth into the septum endymale of the tractus olfactorius ventromedialis initiates a period of rapid proliferation of the neuroblasts there formed, resulting in the development in situ of the nucleus. The subsequent entrance of association fibers via the columna fornicis from the primordium hippocampi is concomitant with its later increase in size.

The first signs of the future lateral choroid plexuses appear at this time as lateral evaginations of the medial wall of the hemisphere where the septum endymale becomes continuous with the ventral leaf of the velum transversum (fig. 24).

The subsequent changes in the larval forebrain are confined to the elaboration of the primordium hippocampi and the choroid plexuses. Two stages in the transformation of the central gray of the pars dorso-medialis into the characteristic scattered cellular arrangement of the primordium hippocampi and the elaboration of the vascular plexus are shown in figures 25 and 26.

DISCUSSION

The above study of the early morphogenesis of the rostral end of the neural tube in *Amblystoma* confirms in large measure the interesting and suggestive theory of Kingsbury concerning the anterior relations of the six longitudinal zones of His. In this form at least the evidence tends to show that the roof and floor plates of His do not meet at the neuropore, nor are the basal and alar plates separated in front of the notochord by the floor plate. Rather, the relations in this region show that the floor plate of His ceases at the fovea isthmi, the midventral portion of the neural plate between the fovea and the preoptic recess being occupied by the rostral continuity of the basal plate and that portion stretching from the preoptic recess to the lamina terminalis by the rostral continuity of the alar plate. In other words, the hypothalamus is made up entirely of basal-plate material and the terminal ridge, eventually occupied by anterior commissure fibers, is derived from the alar plate. In addition, the roof plate of His, instead of reaching ventrally the preoptic recess, stops at the rostral limit of the terminal ridge, the lamina terminalis being formed by the fusion of the lateral lips of the neuropore.

The significance of the above to the theory of the evagination of the hemispheres becomes evident when we consider Herrick's conception of the organization of the walls of the telencephalon. In his 1910 paper he concluded from a study of the morphology of the forebrain in *Amphibia* and *reptiles*, that "each cerebral hemisphere is naturally divided into four parts which correspond respectively with the four primary laminae of the lateral wall of the neural tube whose evagination produced the hemisphere" (p. 498). The lamination so described, while evident in the structurally defined hemisphere, is not so obvious in the stages

preceding and during the evagination of the hemisphere. The wall of a neural tube of a stage-30 larvae just before the beginning of the outpouching does not show clearly the primary lamination of Herrick. It is not until the telencephalic evagination is completely defined (about stage 40) that the four divisions of the cerebral hemisphere become obvious. We may say, then, that the cerebral hemisphere develops as a result of the evagination of the relatively undifferentiated alar plate of the neural tube, cephalad of the sulcus limitans and ventrad of the sulcus diencephalicus medius, and that the lamination subsequently developed is imposed on this primitive arrangement by factors operating through the growth of the embryo. It is evident, then, that the primitive laminae as described by His become to some extent obliterated by the secondary lamination which Herrick has described. In other words, the structural arrangement of the nervous system common to all vertebrates, as His described it, is modified in *Amblystoma* by the acquisition of the further rearrangement described by Herrick.

The fundamental lamination of His is not, however, entirely obscured. Owing to the sharp ventral curvature of the sulcus limitans to end in the preoptic recess, the dorso-ventral lamination (in the sense of His) of the neural tube caudally, becomes antero-posterior in the cephalic portion. Some evidence of the persistence of this arrangement is seen in the fact that a sensory nerve (the olfactory) enters the brain near its cephalic terminus to be followed by a correlation area discharging caudally into the motor area of the hypothalamus, an obvious antero-posterior lamination. But it is likewise just as evident that this antero-posterior relation has given way to, or been dominated by a subsequent dorso-ventral lamination imposed on the fundamental nervous structure. In other words, we find that, following the evagination of the hemisphere, the four primary laminae of Herrick are superimposed at right angles on the primary columns of His in the rostral portion of the neural tube.

One of the interesting facts Herrick discussed in his 1910 paper was the continuity of the columns of the telencephalon with homologous areas in the diencephalon. The two ventral lami-

nae were in practical linear continuity. The dorsal laminae were, however, sharply interrupted by the di-telencephalic groove. The interruption dorsally can be traced to the mechanics of evagination. As was indicated above, the long axis of the telencephalic out-pouching is antero-posterior with the major portion of the evagination resulting from the outward swing of the dorsal margin. This latter movement results largely from the elaboration of the dorsal portion of the hemisphere, more particularly that portion which is to develop into the primordium hippocampi. These factors produce practically no disturbance in the antero-posterior continuity of the basal portions of the brain, but in the dorso-lateral region of the telencephalon result in what is at first a sharp right-angled bend between the dorsal half of the telencephalon and the corresponding area in the diencephalon. This abrupt change in direction of the wall of the neural tube is masked, to some extent, later through the dorso-ventral compression of the whole brain, though the relative interruption in continuity persists.

We may conclude, therefore, that the cerebral hemisphere in *Amblystoma* is evaginated from relatively undifferentiated alar-plate material, which, however, carries with it some evidence of the lamination (in the sense of His) of the more caudal portions of the neural tube, and that superimposed on this primitive arrangement is the secondary lamination (in the sense of Herrick) which characterizes the cephalic portion of the neural tube in older individuals.

The writer wishes to express his appreciation of the very kind and constructive criticism of this paper by Dr. C. J. Herrick, without whose generous assistance the work could not have been accomplished.

SUMMARY

1. The ultimate fate of the ventral boundary of the neuropore in *Amblystoma* larvae is the terminal ridge.
2. The lamina terminalis produced by the fusion of the lateral lips of the neuropore terminates at the terminal ridge.

3. The floor plate of His ends in the fovea isthmi.
4. Between the fovea isthmi and the preoptic recess the mid-ventral portion of the neural plate is occupied by the continuity of the lateral basal laminae.
5. Between the preoptic recess and the lamina terminalis the mid-ventral portion of the neural plate is occupied by the terminal ridge, the continuity of the lateral alar plates.
6. The cerebral hemispheres are evaginated from alar laminae alone and show a cephalo-caudad lamination upon which is imposed the dorso-ventral lamination of Herrick.

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PLATES

ABBREVIATIONS

<i>chr.</i> , chiasmatic ridge	<i>sm.</i> , sulcus diencephalicus medius
<i>cp.</i> , choroid plexus	<i>s. int. ant.</i> , sulcus interencephalicus anterior
<i>di-tel. gr.</i> , di-telencephalic groove	<i>sv.</i> , sulcus angulus dorsalis
<i>emt.</i> , eminentia thalami	<i>tel.</i> , telencephalon
<i>ep.</i> , epiphysis	<i>tr.</i> , terminal ridge
<i>int. f.</i> , interventricular foramen	<i>tr. olf. d. lat.</i> , tractus olfactorius dorso-lateralis
<i>lat. f. b. tr.</i> , lateral forebrain tract	<i>vc.</i> , ventricular cavity
<i>med. f. b. tr.</i> , median forebrain tract	<i>vt.</i> , velum transversum
<i>op. st.</i> , optic stalk	<i>zl.</i> , zona limitans
<i>pa.</i> , paraphysis	
<i>prim. hip.</i> , primordium hippocampi	
<i>rn.</i> , neuropore	

PLATE 1

EXPLANATION OF FIGURES

The figures on this plate are drawn from wax-plate reconstructions of a series of brains of *Amblystoma*. The original models were made at a magnification of 100 diameters and the drawings are reproduced here at $66\frac{2}{3}$ diameters.

13 Lateral view of a model of the brain of a stage-30 (Harrison) *Amblystoma* larva.

14 Medial aspect of the same model.

15 Lateral view of a model of a stage-35 (Harrison) *Amblystoma* larva.

16 Medial aspect of same.

17 Lateral view of a model of a stage-37 (Harrison) *Amblystoma* larva.

18 Medial aspect of same.

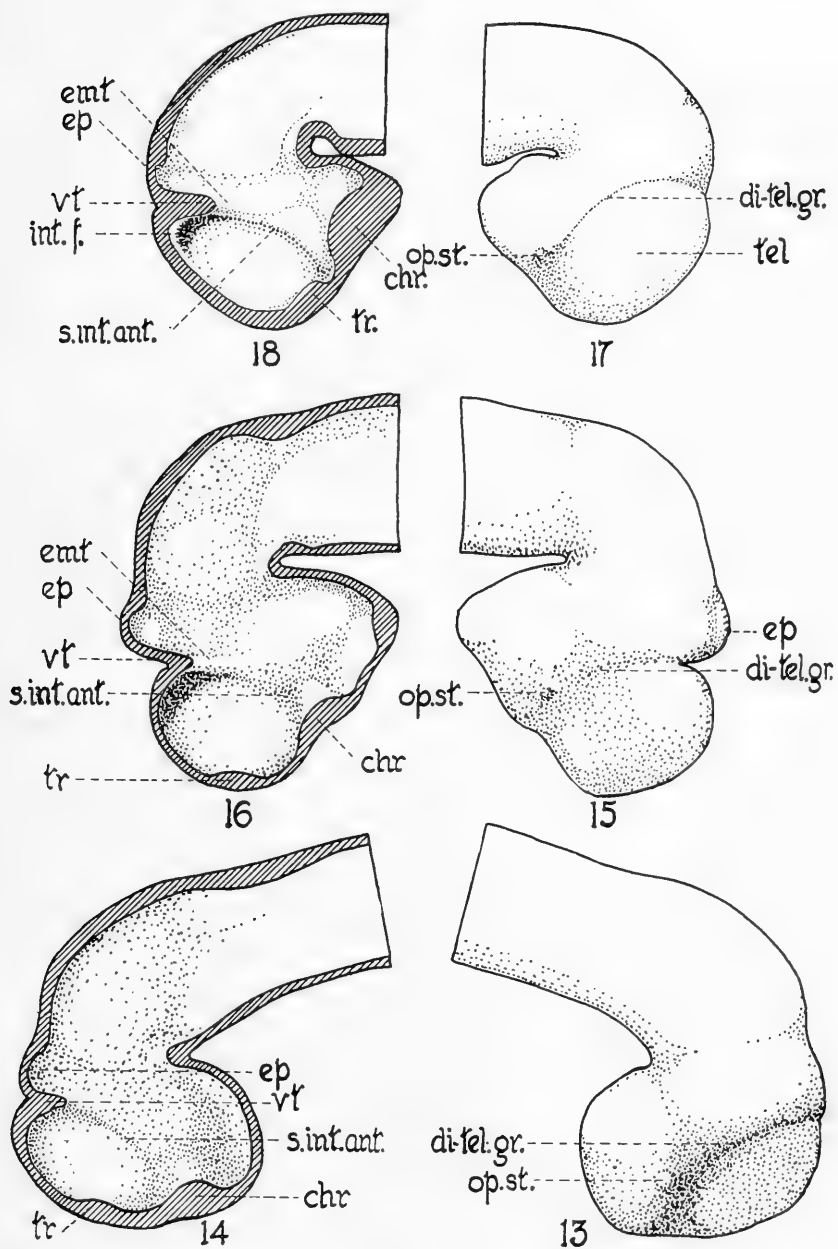


PLATE 2

EXPLANATION OF FIGURES

The figures on this and plate 3 are drawn from transverse sections of a series of brains of *Amblystoma* larvae at a magnification of 100 diameters and reproduced at $66\frac{2}{3}$ diameters.

- 19 Transverse section through brain of a stage-30 *Amblystoma* larva.
- 20 Similar section of a stage-35 larva.
- 21 Similar section of a stage-37 larva just caudad to the velum transversum.
- 22 Transverse section of a stage-40 larva.
- 23 Transverse section of a stage-42 larva just caudad to the velum transversum.

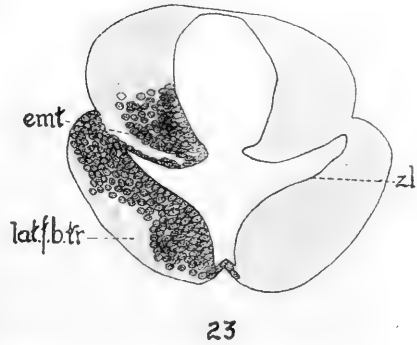
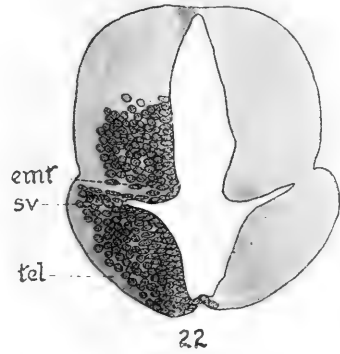
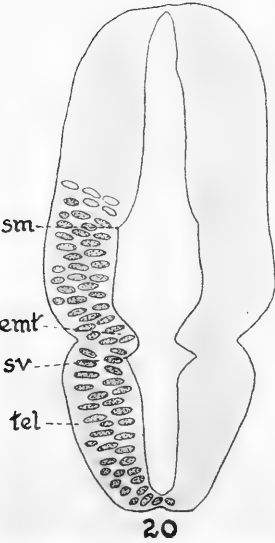
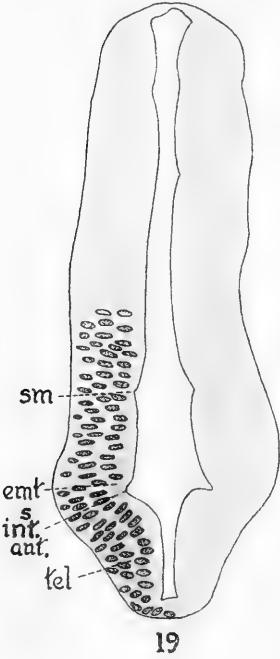


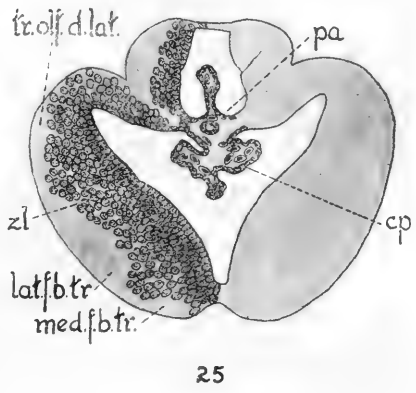
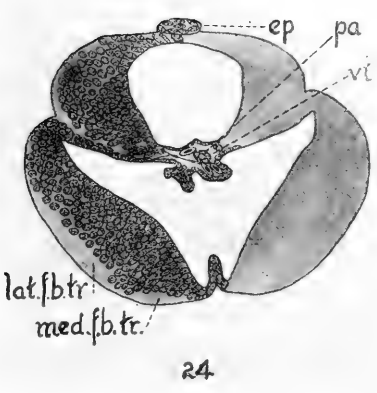
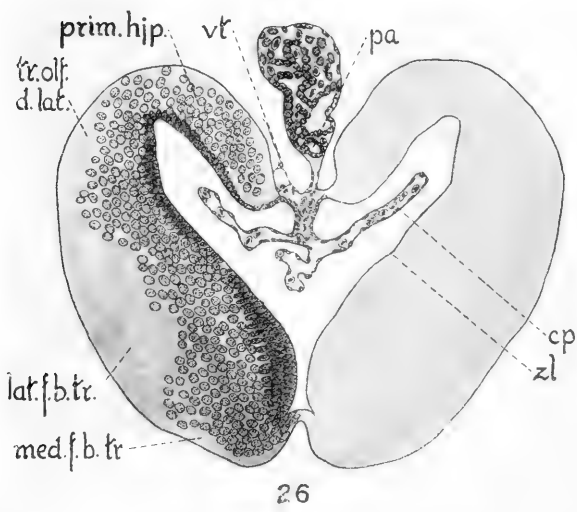
PLATE 3

EXPLANATION OF FIGURES

24 Transverse section of the same brain as shown in figure 23, through the interventricular foramen, showing the velum transversum and the beginning telencephalic choroid plexuses.

25 Transverse section of the brain of a 12-mm. *Amblystoma* larva through the interventricular foramen, showing the appearance of the diencephalic choroid plexus and the elaboration of the telencephalic choroid plexus.

26 A similar section through a three-months-old larva, showing the eventual arrangement of the parts of the hemispheres and the relations of the choroid plexuses.



Resumen por Carl C. Speidel.

Nuevos estudios comparativos en otros peces de células homólogas de las grandes células glandulares irregulares de la médula espinal de la raya.

En la porción caudal de la médula espinal de la raya, *Raia*, existen grandes células glandulares irregulares (células de Dahlgren). Estas células se caracterizan particularmente por la presencia de gránulos de varios tamaños en su vecindad, los cuales representan el producto de su secreción. En la mayor parte de los peces existen células homólogas de estas células de Dahlgren. De los treinta géneros examinados, las células de Dahlgren existen en veintiseis, los cuales incluyen teleósteos, elasmobranquios, y ganoideos. No se han encontrado en los otros grupos animales. En algunas de las formas las células son relativamente pequeñas presentando núcleos casi esféricos o ligeramente lobulados, tal cual ocurre en el bonito, *Scomber scombrus*. En otros peces las células son mayores y presentan núcleo más ramificado, como sucede en el perro de mar, *Mustelus canis*. El tamaño extremo de la célula y la mayor lobulación del núcleo se encuentra en las células del lenguado de verano, *Paralichthys dentatus*, y en la raya, *Raia*. Además de esta última, el autor ha comprobado la existencia de vacuolas y pequeñas cantidades de fino material granular asociado con las células en seis especies de peces. Los gránulos de secreción de gran tamaño, sin embargo, son típicos de las células de la raya. En ninguna de las otras formas examinadas existe evidencia alguna de semejante actividad glandular tan marcada. Las células de Dahlgren de la mayor parte de los peces estudiados se parecen algo a los estados tempranos del desarrollo de semejantes células en los embriones de raya y en las rayas jóvenes. En lo referente al tamaño de la célula, lobulación del núcleo y actividad secretora las células de Dahlgren de la raya representan el tipo más extremo hallado hasta el presente en los peces.

FURTHER COMPARATIVE STUDIES IN OTHER FISHES OF CELLS THAT ARE HOMOLOGOUS TO THE LARGE IRREGULAR GLANDULAR CELLS IN THE SPINAL CORD OF THE SKATES

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TWO PLATES (THIRTEEN FIGURES)

INTRODUCTION

It was pointed out by Dahlgren (1) that there exists in the spinal cord of the skate a series of large remarkable cells of peculiar structure. The complete morphology, location, and distribution of these cells together with some experimental results have been published by the writer in a previous paper (2). This present paper deals especially with the occurrence and appearance in other fishes of cells homologous to these cells of Dahlgren.

In the skate, *Raia*, the cells are present in the anterior horn of gray matter on each side of the central canal, their exact position, however, being rather variable. They are located in the posterior portion of the spinal cord only, being placed in *Raia ocellata* in the posterior half of the tail (i.e., extending from the level of the sixty-fourth vertebra to the tip of the tail, approximately the one hundred and twentieth vertebra).

The cells are of enormous size, a large one that was measured being about 300μ long (antero-posterior measurement), 200μ wide (medio-lateral measurement) and 176μ thick (ventro-dorsal measurement). At first sight of one it is difficult to believe that it is a single cell (fig. 9). It is irregular in outline and contains a huge nucleus of the distributed, branching type extending to all parts of the cell. In sections this often appears to be multiple in character, causing the cell to resemble a fusion of a number of

cells—a syncytium. A study of the embryonic history, however, shows clearly that these cells are not syncytia, but that each is developed from a single cell. The chromatin is scattered throughout the nucleus in the form of large granules. No definite plasmosome is visible. The cytoplasm of a resting cell is homogeneous, but in active stages small and large vacuoles appear. Secretion appears in these and finally the contents of the vacuoles are discharged in the form of granules of various sizes which remain in the tissues of the cord for some time. Often the vacuolar membrane does not break as the secretion material is discharged, but remains intact and holds the enclosed granules together for some time after they have become separated from the secreting cell. A section through the lower part of the spinal cord of the skate in the region of these active cells will be characterized by these deeply staining groups of granules which are distributed chiefly in the ventral portion of the gray matter.

This granular material is protein in character and not easily soluble. It was found that increase in volume of granular material occurred after stimulation of the spinal cord by electricity and pilocarpine, but not after the administration of atropine. The evidence, therefore, both morphological and experimental, indicated that the large cells of Dahlgren in the skate were glandular in nature. The exact function of the secretion, however, was still unknown. It was thought that an extensive comparative study of homologous cells in other fishes might reveal some other forms in which the cells were as unusual as they were in the skate, and that more light might be thrown on the exact nature and function of the cells.

MATERIAL

The fishes were obtained for the most part at Woods Hole, Massachusetts, during the summer months. Thirty genera were studied, comprising most of the common salt-water forms and a few fresh-water ones. These include representatives of the teleosts, elasmobranchs, and ganoids. Examination was also made of the ventral nerve cord of the lobster and horseshoe crab, and of the spinal cord of the lamprey, newt, and mud-puppy. A complete list of the forms examined follows:

List of fishes examined

First group

1. Alewife, *Pomolobus pseudoharengus*
2. Eel, *Anguilla chrysypa*
3. Minnow, *Fundulus heteroclitus*
4. Toadfish, *Opsanus tau*

Second group

5. Butterfish, *Rhombus tricanthus*
6. Catfish, *Amiurus nebulosus*
7. Cunner, *Tautoglabrus adspersus*
8. Dogfish, smooth, *Mustelus canis*
9. Dogfish, spiny, *Squalus acanthias*
10. Garpike, *Lepidosteus osteus*
11. Goosefish, *Lophius piscatorius*
12. Mackerel, *Scomber scombrus*
13. Menhaden, *Brevoortia tyrannus*
14. Puffer, *Sphaeroides maculatus*
15. Sand-dab, *Bothus maculatus*
16. Sandshark, *Carcharias littoralis*
17. Sead, *Decapterus* sp.
18. Sculpin, *Acanthocottus* sp.
19. Scup, *Stenotomus chrysops*
20. Sea-robin, *Prionotus carolinus*
21. Shark-sucker, *Echeneis naucrates*
22. Swordfish, *Xiphias gladius*
23. Sting-ray, *Dasyatis* sp.
24. Tomcod, *Microgadus tomcod*
25. Torpedofish, *Torpedo* sp.
26. Tautog, *Tautoga onitis*
27. Whiting, *Melucius bilinearis*
28. Winter flounder, *Pseudopleuronectes americanus*

Third group

29. Summer flounder, *Paralichthys dentatus*
30. Skate, *Raia*
 - Barndoor skate, *Raia laevis*
 - Italian skate, *Raia punctata*
 - Spiny skate, *Raia radiata*
 - Summer skate, *Raia erinacea*
 - Winter skate, *Raia ocellata*

Other forms examined

- Horseshoe crab, *Limulus polyphemus*
- Lobster, *Homarus americanus*
- Lamprey, *Petromyzon* sp.
- American newt, *Diemyctylus viridescens*
- Mud-puppy, *Necturus* sp.

OBSERVATIONS

For purposes of description the fishes may be conveniently divided into the three groups indicated in the list above. In the first group cells homologous to the Dahlgren cells are lacking. In the second group the cells are present and are of moderate size, though varying somewhat. In the third group the cells are enormous and highly modified.

In regard to the fishes of the first group, the alewife, eel, minnow, and toadfish, I know of no special reason why the cells of Dahlgren should be lacking. I have already suggested that they may be looked upon as having been derived from modified embryonic nerve tissues. A study of their development in skate embryos shows that they are derived from the same cells which give rise to nerve tissues. In these four fishes it would seem that there had never been any modification of the embryonic nerve tissues in the direction of the Dahlgren cells in this region, or so slight a modification that it is not easily noticeable.

The second group varies somewhat with reference to the Dahlgren cells, both in size and appearance. In forms like the mackerel and goosfish the cells bear some resemblance, perhaps, to nerve cells. They are larger than nerve cells, but still relatively small. One cell of about average size in the mackerel measured 60μ in length, 52μ in breadth, and 39μ in thickness. The cell processes are not numerous. The nucleus is usually only slightly lobulated, and may be almost spherical. One or more plasmosomes may be distinguished, the number often depending upon the number of lobes of the nucleus. In each of the cells of figures 1, 2, 3, and 4 taken from the mackerel one plasmosome is present (although in figure 2 it is not shown in the section represented). In the cell shown in figure 5 taken from the butterfish two plasmosomes are present. In one cell from the goosfish, the nucleus of which had three lobes, three plasmosomes were distinctly present, one in each lobe. Other forms, such as the catfish, cunner, garpike, menhaden, butterfish (fig. 5), puffer, sea-robin, scad, scup, shark-sucker, tautog (fig. 6), tomcod, and whiting, show somewhat greater lobulation of the nucleus, plasmosomes sometimes being present, sometimes not. The cell-body is also larger in these forms.

The cytoplasm of the cells in most of the forms was homogeneous. In the cell from the sandshark (fig. 7), however, there may be seen at the periphery a vacuole containing a fine granular precipitate. This is probably a secretion of the cell. Well-defined granular precipitate of secretion contained in definite vacuoles at the periphery of the cell was also seen in the shark-sucker. It has previously been pointed out that in the Dahlgren cells of the skate such vacuoles containing fine precipitate are of common occurrence. These vacuoles often are outside of the cell in the neighboring tissues of the spinal cord. Granular material associated with the cells, but outside of them, has been noticed in the dogfish, flounder, menhaden, and possibly tautog. Another interesting feature in connection with the appearance of the cytoplasm of the Dahlgren cells is that it is often pierced by one or more capillaries (especially in the case of the shark-sucker), so that in cross-section the capillary with its endothelium and enclosed blood corpuscle appears to lie within the cytoplasm of the cell.

In the case of the other fishes of the second group, the sting-ray (fig. 8), the dogfish, and torpedo-fish, the cells are larger, the volume of nuclear material relatively larger in comparison to the amount of cytoplasm and the nucleus more lobulated. The cells in these forms, indeed, almost make a series of transition stages leading up to the third group. In general, it may be said that the Dahlgren cells are larger and their nuclei more highly branched in the elasmobranchs than in the teleosts or ganoids. The one notable exception to this rule is the summer flounder. In none of the cells of the second group were Nissl bodies seen, although in the neighboring muscle motor nerve-cells they could be clearly made out.

The cells from the fishes of the second group, as arranged in figures 1 to 8, make an interesting comparison with the cells in embryo skates in various stages of development. The two series are very similar. In other words in the development of the complex type found in the adult skate simpler stages are passed through. These simpler stages may be found in the adults of the fishes which have been placed in the second group.

The third group includes only the flounder and the skate. The cells in these two forms are the most highly modified in size and differentiation yet found. The skate cells have already been described and a typical cell is shown in figure 9. This cell as seen in a cross-section of the spinal cord is elongated in two directions (ventro-medially and laterally), and at each of the ends is seen a number of granules, the secretion material of the cell. If the serial sections passing through this cell be followed, first in an anterior direction and then in a posterior direction, it will be found that the number of granules and volume of granular material both increase. The volume of granular material is greater at the anterior and posterior ends of the cell than at the middle region. The amount shown in the figure, therefore, represents only a small percentage of the total amount associated with the cell (much less than a tenth). The skate differs from all other forms that have been examined in the presence of large amounts of this granular material associated with the cells.

Besides the skate, the flounder is perhaps the most interesting form. In this animal the spinal cord terminates posteriorly in an enlargement, the terminal enlargement, which extends anteriorly for a short distance, forming a sort of cap over the dorsal part of the spinal cord. In this region, and for about an inch anteriorly, the spinal cord instead of appearing macroscopically as glistening white in color appears as a rather transparent gray. It is here that the Dahlgren cells are most numerous and of greatest size, the gray color of the spinal cord being due to their presence together with the lack of many myelinated nerve fibers.

The cells are very large, about as large as in most species of skates. In the terminal portion of the spinal cord they are present in great numbers closely grouped together so that in both longitudinal and transverse sections they easily form the most conspicuous part of the section (figs. 12 and 13). A characteristic collection of the Dahlgren cells, as seen in longitudinal section, is shown in figure 12, a low-power microphotograph. It is interesting to note that in many of the cells in this section the nuclear material is located not at the center of the cell, but at the periphery. For this reason, the cell looks somewhat like a

syncytium, bearing a superficial resemblance at least to the giant-cells which occur in certain diseases (as in miliary tuberculosis) that have been formed by a fusion of many endothelial cells. Figure 13 is a low-power microphotograph showing the general appearance of a cross-section of flounder spinal cord near the posterior end. Six or more of the large Dahlgren cells may be seen. The terminal enlargement appears here as a large cap on the dorsal side of the spinal cord proper. The central canal is located near the ventral side. The horns of gray matter are not well defined this far posterior in the spinal cord. In the terminal enlargement may also be seen two or three small Dahlgren cells, or pieces of cells. The terminal enlargement is really a continuation of the spinal cord (although in this cross-section it appears to be separated from it), but it contains practically no gray matter. These cells or pieces of cells, therefore, which are included in it seem rather out of place.

As may be seen from the figures, the cells are enormous. In actual size a fairly large flounder cell was found to measure 240μ ventro-dorsally, 160μ antero-posteriorly and 110μ medio-laterally. The average-sized cell is somewhat smaller than this. The details of structure of a typical cell may be seen in the cell shown in figure 10. The similarities in appearance between this cell of the flounder and the one of the skate are evident. The cells of the flounder, however, seldom have either vacuoles or granules associated with them, at least in appreciable amounts. In some cases I have found vacuoles near the edge of a cell containing a fine granular, or threadlike, or rod-like precipitate or secretion which apparently has been elaborated by the cell. A typical one of these is shown in figure 11. Similar thread-like or fine granular secretion in vacuoles either inside or outside the cell is also often found in the case of the skate cells. In the flounder, however, in spite of the huge size of the cells, I have never seen any of the large-sized granules similar to those which form such a characteristic part of the secretion of the cells of the skate.

The observations of the cells of the flounder, then, indicate that in spite of their enormous size they do not elaborate any appreciable amount of granular material, as is the case in the

skate. It was thought that it might be of interest in this connection to see whether or not the administration of pilocarpine or stimulation with electricity would have any effect in stimulating the cells. Accordingly, a solution of pilocarpine hydrochloride (0.1 per cent) was injected into each of four flounders by way of the peritoneal cavity and by way of the vertebral canal. This was allowed to act for from ten to fifteen minutes. Examination of these animals showed that no marked increase in granular secretion or in vacuole formation had taken place. Electrical stimulation of the spinal cord of each of four animals gave a similar result. If the Dahlgren cells of the flounder are at all glandular in nature they are certainly very sluggish, far more so than the homologous cells in the skate.

It has already been pointed out in a previous paper (2) that the conclusion that the cells of Dahlgren in the skate were of glandular nature applied only to the skate. At that time only a few kinds of fishes had been examined and the appearance of homologous cells in these forms did not warrant the conclusion that they were glandular in these other forms. It seems to me that the structure of the cells from a comparative standpoint suggests a series of transition stages from primitive nerve tissue to glandular tissue. In the skate the cells are markedly glandular. In the cells of the barndoor skate, *Raia laevis*, a very large amount of granular secretion is elaborated; in the winter skate, *Raia ocellata*, and the Italian species, *Raia punctata*, there is not quite so much produced; while in the spiny skate, *Raia radiata*, and the summer skate, *Raia erinacea*, still less secretion material is to be found. In the flounder, shark-sucker, dogfish, sandshark, and menhaden the cells seem to have only a slight glandular activity. In the other forms no secretion material at all was seen.

Examination was also made of the spinal cord of the lamprey, *Petromyzon*, the American newt, *Diemyctylus*, the mud-puppy, *Necturus*, and of the ventral nerve cord of the lobster, *Homarus*, and the horseshoe crab, *Limulus*. In none of these were cells found that were homologous to the Dahlgren cells of the fishes.

SUMMARY

1. Cells homologous to the large irregular glandular cells of Dahlgren in the skate are present in the large majority of fishes. Of thirty genera examined the Dahlgren cells were found in twenty-six. They have not been found in any other group of animals. They are always located (when present) in the posterior portion of the spinal cord.

2. Morphologically, many gradations are found. In some of the fishes the cells, though somewhat larger, still bear some resemblance to nerve cells, the nucleus being almost spherical or slightly lobulated and containing one plasmosome, as in the mackerel. In other fishes, as in the goosfish and butterfish, the nucleus is more lobulated with two or three plasmosomes often present. In others, as in tautog, sandshark, sting-ray, and dogfish, the cells are larger and the nucleus still more lobulated. In some a few vacuoles containing precipitate or secretion are sometimes found associated with the cells. The extreme size is reached in the cells of the flounder and skate. In addition to the enormous size of cell and nucleus, a large amount of granular secretion is also usually present in the skate with occasional vacuolation of the cytoplasm. In the flounder a very few vacuoles containing granular or thread-like precipitate may be found occasionally associated with the cells.

3. The cells have been found in elasmobranchs, teleosts, and ganoids. They are relatively larger in elasmobranchs than in teleosts (with the exception of the summer flounder) and ganoids.

4. Vacuoles and secretion material are not present to any great extent in any of the fishes other than the skate. Small amounts of fine granular material have been found associated with the cells of the flounder, sandshark, shark-sucker, scup, menhaden, and dogfish. Secretion granules of large size, however, are typical of the skate cells alone. In none of the other forms, therefore, is there evidence of marked glandular activity.

5. In size of cell, lobulation of nucleus and secretory activity the Dahlgren cells of the skate represent the most extreme type

found in any of the fishes. The Dahlgren cells of most of the fishes examined resemble very much the early developmental stages of the Dahlgren cells in skate embryos and young skates.

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PLATES

ABBREVIATIONS

c.c., central canal
gr., granular secretion
nuc., nucleus
pl., plasmosome

sp. c., spinal cord proper
ter. en., terminal enlargement
vac., vacuole
D. c., Dahlgren cell

PLATE 1

EXPLANATION OF FIGURES

All of the figures of plate 1 were drawn with camera lucida to the same scale of magnification ($\times 390$).

1 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus is almost spherical and a plasmosome is present. The diameter of this cell is about twice that of an ordinary muscle motor nerve-cell in the same animal. This cell and the cells of figures 2, 3, and 4 were all present in the same cross-section.

2 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus is slightly elongated and lobulated. A plasmosome is present, but is not shown in the section represented.

3 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus has three lobes.

4 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. This section shows the nuclear material enclosing a central core of cytoplasm.

5 Dahlgren cell from the spinal cord of the butterfish, *Rhombus tricanthus*. The nucleus is more highly branched than in the mackerel, and in this section it appears to be multiple. Two plasmosomes may be seen. The size of the cell is also somewhat larger than in the mackerel.

6 Dahlgren cell from the spinal cord of the tautog, *Tautoga onitis*. Note the two greatly elongated nuclear masses. These are, however, in reality parts of a single nucleus.

7 Dahlgren cell from the spinal cord of the sandshark, *Carcharias littoralis*. Note the increase in lobulation of the nucleus. Note also the vacuole at the edge of the cell containing some fine granular material. Apparently the vacuole has just burst and discharged most of its contents. Plasmosomes are not distinguishable in this cell nor in any of the cells of the succeeding figures.

8 Dahlgren cell from the spinal cord of the sting-ray, *Dasyatis*. This shows further increase in size of the cell and size and lobulation of the nucleus.

9 Dahlgren cell from the spinal cord of the skate, *Raia ocellata*. Both the cell body and nucleus are of enormous size. Note especially the characteristic groups of granules of various sizes at the ends of the cell. Each group of granules represents the original content of a single vacuole. Only a small percentage of the granular secretion is shown in the section represented, most of it being present in the sections near the ends of the cell (anterior and posterior ends).

10 Dahlgren cell from the spinal cord of the summer flounder, *Paralichthys dentatus*. The size of the cell is about the same as that of the skate, *Raia ocellata*. No large granules, however, have ever been found associated with these cells of the flounder, such as are present in the skate.

11 Typical vacuole sometimes found associated with the cells of the summer flounder, *Paralichthys dentatus*. This vacuole contains a fine granular or thread-like precipitate which is apparently elaborated by the cell. It is similar in appearance to the vacuoles containing fine secretion material which are often found associated with the Dahlgren cells of the skate (both inside the cell and outside).

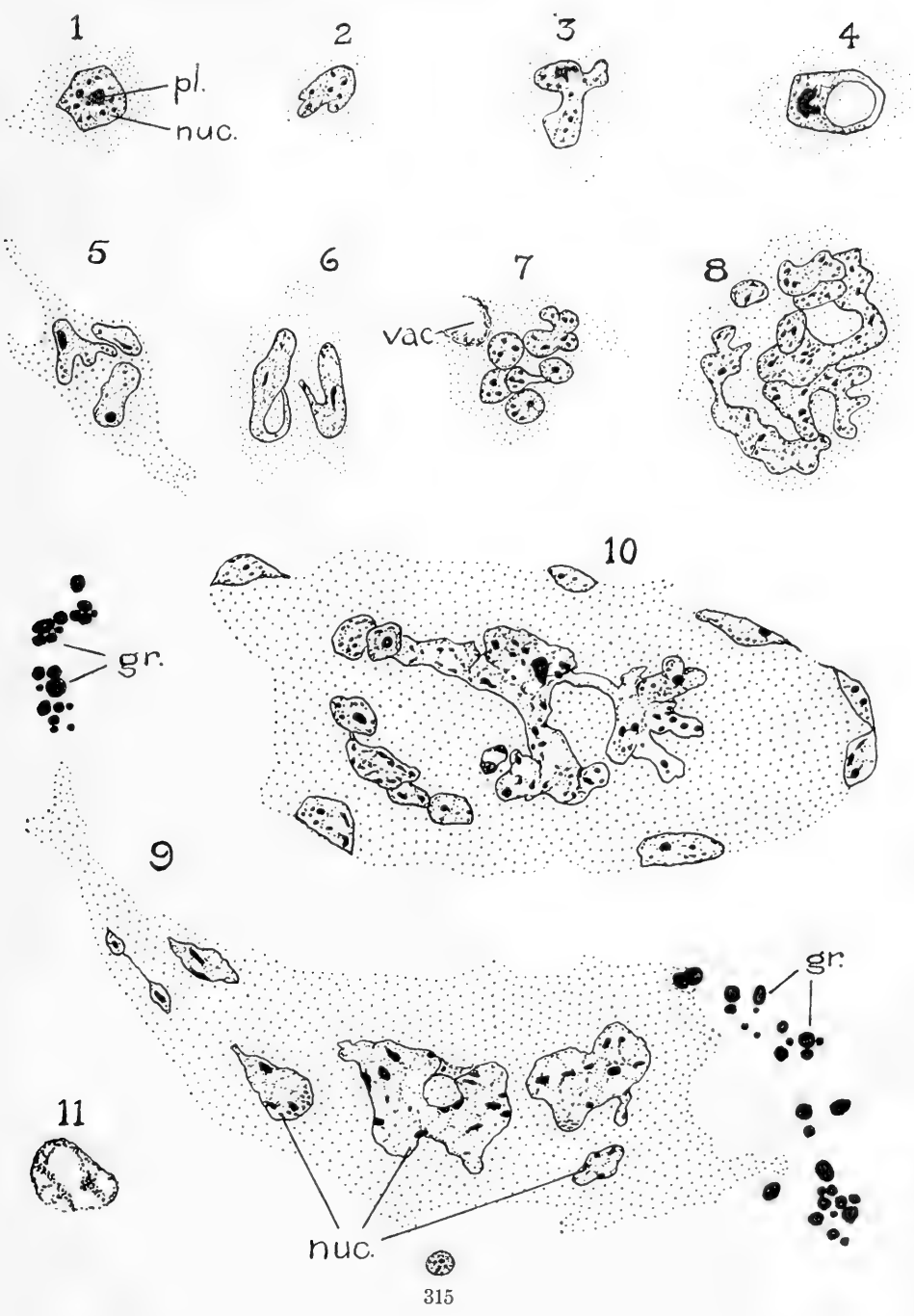
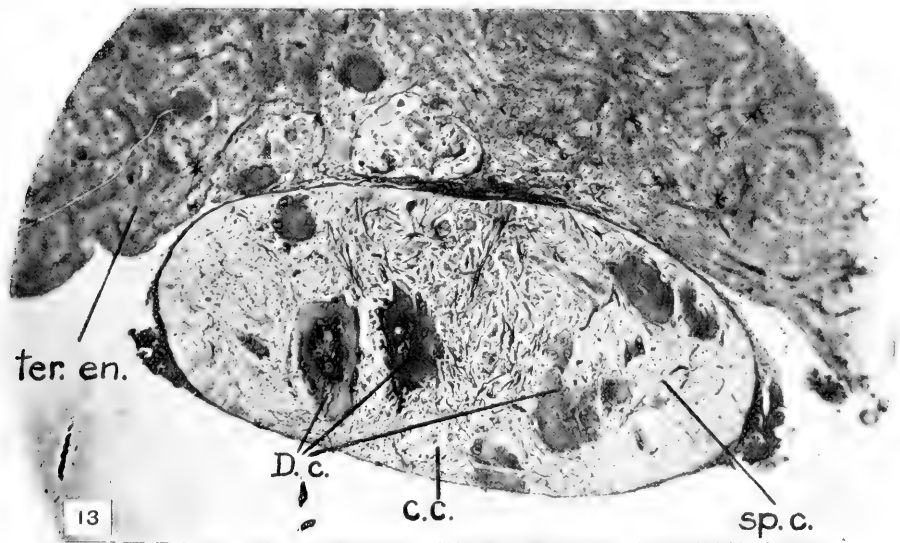
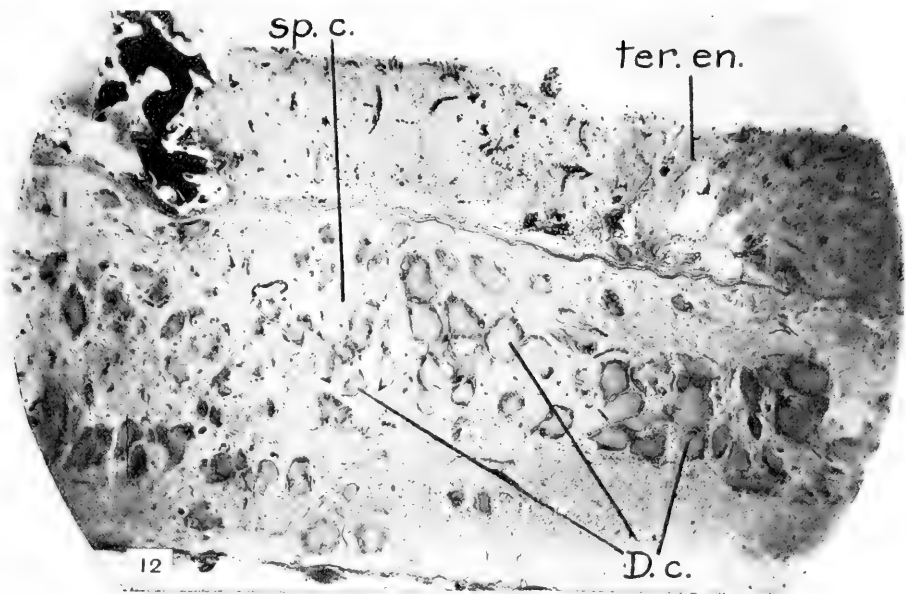


PLATE 2

EXPLANATION OF FIGURES

12 Longitudinal section of the posterior terminal portion of the spinal cord of the summer flounder, *Paralichthys dentatus*. Note the large collection and the great size of the Dahlgren cells in this region. In many of the cells the nuclear material may be seen peripherally arranged about a central core of cytoplasm. The 'terminal enlargement' of the spinal cord appears here as a cap over the dorsal side of the spinal cord proper. Low-power microphotograph. $\times 60$.

13 Cross-section of the spinal cord of the summer flounder, *Paralichthys dentatus*. The 'terminal enlargement' appears here as a cap on the dorsal side. Five or more of the large Dahlgren cells may be seen in the spinal cord proper. A couple of small Dahlgren cells or pieces of cells may be seen in the 'terminal enlargement.' The Dahlgren cells occupy about one-quarter of the cross-section area of the spinal cord. Low-power microphotograph. $\times 70$.



Resumen por el autor, Roy L. Moodie.

La influencia del sistema de la línea lateral sobre los elementos óseos periféricos de los peces y anfibios.

Los órganos sensoriales de la línea lateral no son tróficos para el esqueleto de la cabeza. La asociación de los canales de la línea lateral con ciertos elementos óseos periféricos es un rasgo constante en la organización de los peces y anfibios desde los tiempos del Devónico hasta el presente. La presencia de tejido conectivo denso en los canales de la línea lateral del cuerpo y de la cabeza es importante porque suministra una base inactiva para el depósito de material óseo. En la cabeza de *Amiurus* existen dos complejos de huesos que se desarrollan de dos modos diferentes por las siguientes causas: a) Por la influencia poderosa de la presión, que es la más importante, y b) por la influencia mecánica de los canales de la línea lateral.

La naturaleza de la influencia de los canales de la línea lateral sobre la formación de los elementos óseos periféricos es mecánica porque suministra una substancia inactiva bajo la forma del tejido conectivo denso que forma los canales. Ofrecen una base más altamente organizada de material coloide para la atracción del depósito de material calcáreo a causa de su desarrollo precoz y a causa de la influencia mecánica de la superficie y los rasgos de los capilares presentes en la superficie de los canales. Las primeras espículas óseas se depositan en dirección paralela a los canales; son de contorno sinuoso e irregulares, debiéndose esta irregularidad a una respuesta a la forma de los osteoblastos irregularmente dispuestos entre los cual es se depositan los filamentos. Estos últimos son granulares y no cristalinos.

THE INFLUENCE OF THE LATERAL-LINE SYSTEM
ON THE PERIPHERAL OSSEOUS ELEMENTS
OF FISHES AND AMPHIBIA¹

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FIVE FIGURES

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INTRODUCTION

The present study is the result of an attempt to determine whether the lateral-line system, in its development, initiates the formation of bone. The trophicity of the nervous system in bone growth and bone pathology is well known, having been discussed by Abraham ('99), Cassirer ('10), Luciani ('15), Moral ('19), and Nasse ('80), and it seemed to me possible that the sense organs of the lateral-line system might prove to be trophic for the skeleton of the head especially. This idea was supported by the acknowledged fact that the lateral-line grooves on the peripheral osseous elements of fishes, both ancient and modern, and in ancient amphibians furnish an important criterion in homologizing the cranial elements.

¹ Aided by grants from the Elizabeth Thompson Science Fund.

Sagemahl's ('91) objection to the value of this system as a criterion of homology in the skull of the Cyprinidae has been satisfactorily explained by Allis ('04), who, on an examination of three genera of this family, learned that "it is the parietal bones of these fishes, and not their supratemporal canals, that are not the homologues of those in the other fishes." Allis in the same paper goes on to discuss and figure, in twelve plates, the relationship of the latero-sensory canals to definite bones of the cranium as established in twenty-one genera in fishes.

The value of the canals in homologizing the bones of ancient Amphibia and fishes the writer has likewise pointed out, and there is a firm basis for stating that this system of canals is one of the most constant features of primitive vertebrate organization.

The antiquity of such an arrangement as seen in the heads of modern fishes is suggested by the condition of the latero-sensory canals described by Bryant ('19) in *Eusthenopteron foordi* from the upper Devonian rocks of Scaumenac Bay, Quebec. The nature of the sensory canals of the head among the Triassic fishes of Spitzbergen has been discussed in an especially fine way by Erik A. Son Stensiö ('21). This plan (fig. 1) outlined so early in vertebrate organogenesis has since suffered little change.

Unfortunately, the skulls of modern Amphibia are seldom cut by the latero-sensory canals, but we have the work of Harrison ('04) to show that the lateral line of amphibians is of taxonomic importance, and I have shown ('16) that the lateral-line system as preserved on Carboniferous Branchiosauria is essentially like that of the modern urodeles, especially *Necturus*.

LITERATURE

Such being the condition of our knowledge of the relationship of the bones of the head and the latero-sensory canals, I undertook to determine if the cranial bones developed in response to the sense organs or to the canals after they were fully supplied with the sensory endings. The only literature which has a direct bearing on the problem is the study of Klaatsch ('94), who has discussed, in connection with many other problems,

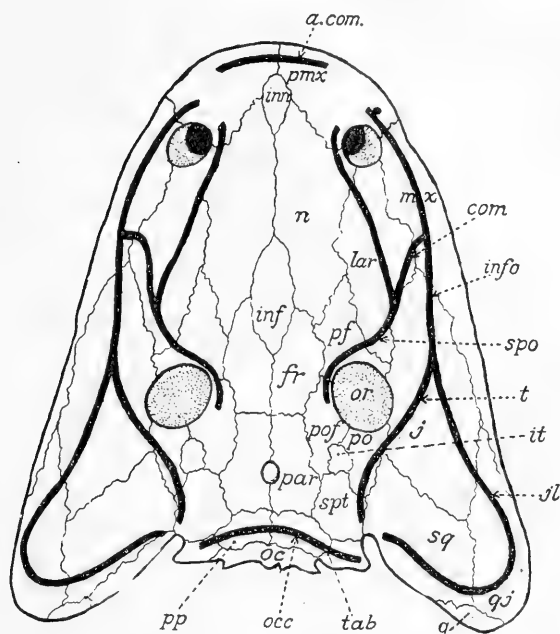


Fig. 1 Generalized figure of dorsum of an early amphibian skull, to show position of elements of cranium in relation to lateral-line canals. The outline is based on that of *Eryops*, but does not indicate that form.

ABBREVIATIONS

a. com., anterior commissure of lateral-line canals

com., commisural communication between infra- and supra-orbital canals

fr., frontal bone

inf., interfrontal

inn., internasal

info., infra-orbital lateral-line canal

it., intertemporal

j., jugal

jl., jugal lateral-line canal

lar., lacrimal

max., maxilla

n., nasal

oc., occiput

occ., occipital cross-commissure of lateral-line system

or., orbit.

par., parietal

po., postorbital

pf., prefrontal

pof., postfrontal

pmx., premaxilla

pp., postparietal

q., quadrate

qj., quadratojugal

spo., supraorbital canal

sq., squamosal

spt., supratemporal

t., temporal lateral-line canal

tab., tabulare

"the first formation of bone in relation to the sensory organs of the skin," although the problem has been touched by a number of investigators, notably Schleip ('04), who discussed the origin from the ectoderm of the osteoblasts forming the bones surrounding the latero-sensory canals, thus indicating a double origin for the bones of the head. Schleip does not, however, entirely agree with Klaatsch ('95) regarding the origin of the osteoblasts from the ectoderm, maintaining that from the crowding of the cells it is difficult to determine their origin. Concerning the influence of the latero-sensory canals in stimulating the 'skeroblasts' to activity, Schleip has little to say, but Klaatsch's work shows that for the peripheral bones the lateral-line system does have an influence. The classic papers of McMurrich ('84) and Wright ('84) were also studied and were found to be of great value, as attested by the references in the following pages.

MATERIAL

The problem was first attacked on fresh living material at Woods Hole. Here the embryos and larvae of various species of fishes were studied under the polariscope, which had previously been found useful by other investigators in determining the growth of the skeleton in echinoderm larvae. Vertebrate material, however, studied in this manner proved very unsatisfactory.

Pigment was the chief cause of confusion and the density of the tissues was another. Spicules of bones are occasionally evident and their relation to the canals can be seen, but in a very unsatisfactory manner.

After some deliberation it was decided to undertake the study of the problem in the young *Amiurus*. While the work was in progress, great aid was rendered by the appearance of the excellent study of Kindred's ('19) dealing with the development of the skull of this genus. The first step in the undertaking was to determine at what age the skull bones began to develop. This was done by clearing in KOH,² according to the Schultze method,

² This method has been elaborated by Mall, *Am. Jour. Anat.*, vol. 5, pp. 433-458, 1906, and by the writer in the same journal, vol. 7, p. 433, 1908.¹ The chief advantage of the method is that one is enabled to determine the earliest appearance of the spicules of bone which are often overlooked in histological sections.

a graded series of specimens ranging in length from 12 mm. to 60 mm. The desired stages were found between 18 mm. and 35 mm. This having been determined, the necessary stages were set aside for sectioning, and study of the cleared specimens began.

Since the results of this study are rather complex, being an attempt to correlate the facts of development, adult anatomy, and paleontology, the following account is divided into, 1) actual observations on *Amiurus*, 2) suggested explanations of osteogenesis, and, 3) their application to paleontology.

THE BODY CANALS

While my study is chiefly restricted to a determination of the conditions in the head, yet it will be of great importance to state that the body lateral-line canals are often, in fact, usually, associated with dense connective tissue which may become ossified. The association of the sense organs of the skin of the body with dense connective tissue in *Amiurus* is shown in figure 5.

The canals, though frequently composed of dense connective tissue (fig. 5, *c.t.*) or cartilages, are often highly differentiated and are referred to as 'drain-pipe bones,' a term applied to the suborbital chain of bones in the head. Wright ('84, p. 263) has given an interesting account of the histology of the body canals of *Amiurus*, contrasting them with the lateral-line canals of the head (*ibid.*, p. 264). The condition of the sense organs in relation to the cartilage of the body canals has been described by Latimer for *Polyodon* ('19) and by many other writers for various fishes. What influence the lateral-line sense organs have upon the development of the heavy material of the body canals has never been determined, so far as I am aware, and since it is outside the scope of this investigation it must be left for future study.

NATURE OF THE HEAD SKELETON OF AMIURUS

It was quite evident from an examination of specimens of about 20 mm. in length that there were two or more sets of bones in the head of this animal, as had already been suggested by the work of Schleip and Klaatsch in other fishes.

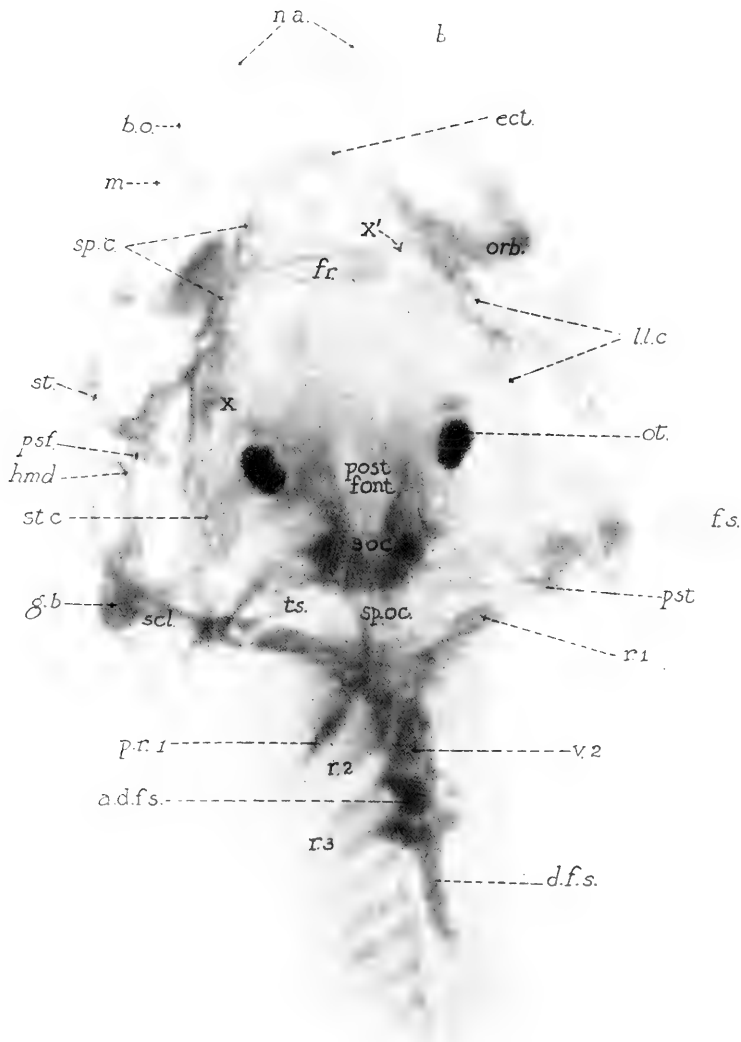


Figure 2

McMurrich ('84, p. 279) gives a more elaborate account of the nature of the head skeleton of *Amiurus*, but agreeing with the above conclusions. He, too, based his statements on a 20-mm. larva of *Amiurus* which he had studied histologically.

The bone which first appears in the head is the supraoccipital, which with its heavy spina occipitis is a very evident element in the cleared specimen at this stage. It is clear that its origin is in no way involved in the latero-sensory canals, the plan of which is already laid down as shown by the photograph (fig. 2) in direct sunlight under a binocular of a specimen 24.2 mm. long. The sphenotics arise in relation with the lateral-line system and are in a definite way influenced by it. Thus toplicity of the lateral-line system for this particular bone is suggested. The impulse which initiates the development of the skull bones is a deep-seated factor in determining the time of origin of many of the osseous elements.

Fig. 2 Photograph of a dorsal view of the anterior part of body of a young *Amiurus* (24.2 mm., measured from tip of caudal fin), showing the nature and degree of ossification of the skeleton of the head and girdle. The ossification, segmental in nature, along the supraorbital lateral-line canal is especially noteworthy.

ABBREVIATIONS

<i>a.d.f.s.</i> , anterior dorsal fin spine	<i>post.font.</i> , posterior fontanelle
<i>b.</i> , barbel	<i>r. 1.</i> , greatly expanded first parapophysis
<i>b.o.</i> , ossification in base of barbel	<i>r. 2.</i> , rib, of second vertebra
<i>d. f. s.</i> , posterior dorsal fin spine	<i>r. 3.</i> , rib, of third vertebra
<i>ect.</i> , ectethmoid	<i>scl.</i> , supraclavicle
<i>fr.</i> , frontal, ossification of the transverse bar	<i>soc.</i> , supra-occipital
<i>f. s.</i> , fin spine of pectoral fin	<i>sp. oc.</i> , spina occipitis
<i>g. b.</i> , clavicle forming girdle base	<i>sp. c.</i> , supra-orbital lateral-line canal
<i>hmb.</i> , hyomandibular	<i>st.</i> , subtemporal lateral-line canal continuous with mandibular canal
<i>l. l. c.</i> , right lateral-line canal	<i>st. c.</i> , supratemporal lateral-line canal
<i>m.</i> , mandible	<i>ts.</i> , transscapula
<i>n.a.</i> , nasal	<i>v. 2.</i> , second vertebra
<i>orb.</i> , orbit	<i>x.</i> , posterior expansion of supraorbital canal
<i>ot.</i> , otolith	<i>x'</i> , point of influence of lateral-line canal on primary ossification of frontal bone
<i>p.r.1.</i> , posterior expansion of first parapophysis	
<i>psf.</i> , postfrontal	
<i>pst.</i> , posttemporal	

Suggested explanations for this are given later on. The lateral peripheral osseous elements appear much later than the supra-occipital and especially is this true of the suborbital chain of bones, to the development of which the lateral-line system is so intimately related. Our knowledge of the nerves and sense organs of the lateral-line system of *Amiurus* is adequate, the subject having been discussed by Wright ('84) and Herrick ('01) and further developed in other siluroids by Allis ('04).

CONDITION OF THE HEAD SKELETON OF AMIURUS AT 24 MM.

Returning now to a consideration of the bones of the head in their relation to the lateral-line canals in *Amiurus*, reference may be made to figure 11 in Kindred's ('19) paper to show this association. He figures an advanced stage after the formation of both sets of bones. As I have previously stated, the set least associated with the lateral-line system is the first to develop. The distinctness of the supra-occipital from the lateral-line bones is indicated by Kindred's ('19) statement: "There is no lateral line ossification anywhere near the vicinity of the developing supra-occipital." This is shown in figure 2. The supra-occipital later grows out in contact with the lateral-line canal and is modified by it. McMurrich ('84, p. 271) states, "it (the supra-occipital) presents (in the adult) many minute foramina, belonging to the system of the mucus canals."

The first bones in the head may develop in response to stress, for they are seen (fig. 2) in the occipital region at the point where the head joins the body. The first vertebra with its large parapophysis (fig. 2, *r.1.*) is extensively ossified, as are likewise the dorsal spines. The elements of the pectoral girdle are also easily identified, though they have not all developed equally. The great advance in ossification shown by the frontal is difficult to understand, since it is not subjected to strain and only in part to the direct influence of the lateral-line system. All other dorsal cranial elements except the supra-ethmoid and all lateral ones with the exception of the hyomandibular are developed in response to some influence exerted by the lateral-line canals.

The relation of the canals to the early deposition of osseous material is shown in figures 3 and 4. Here the bony substance is deposited in spicules and in granules immediately adjacent to the circumference of the lateral-line canals. Deposition of bone is not brought about by nervous stimulus arising from the lateral-line end organs. The sense organs of the supra-orbital canals (fig. 3), if they exist at all at this point, are enclosed in bone, and are thus not in relation with the osteogenic areas. The fact that the ossification around the supra-orbital canals is segmental in the larva suggests the presence of tubules and dermal pores in the young which are lost in the adult.

Regarding the later development of the osseous tubes around the lateral-line canals, McMurrich ('84, p. 279) states "the osseous tube does not remain distinct but fuses with the subjacent bone, whether formed in membrane or perichondrally."

CONDITION OF THE SKULL BONES IN AMIURUS AT 50 MM.

Nearly all the cranial elements are fully established at this stage, excepting the nasal, the lacrimal, and the suborbitals, which are lateral-line canal bones. The ossification of the skull in *Amiurus* is the reverse of the usual antero-posterior embryonic development, since the posterior cranial elements are the first to be fully established.

The lacrimal is represented as a triradiate mass of aggregated spicules arranged around the anterior termination of the sub-orbital canal. The nature of the ossification is intermediate in character between that of the supraorbital canal, where it is in threads, and the suborbital chain, where it is in granules. The nasal, formed around the anterior end of the supraorbital canal, is composed of spicules arranged around the canal. The suborbital chain of bones is still undifferentiated and the condition of the osseous material is shown in figure 4. The supraethmoid is fully formed, and has been developed apparently entirely independent of any influence from the lateral-line system. The course of the canals through the sphenotics and frontals is clearly evident, due to a dense deposition of calcium in granular form on the walls of the canals. The osseous tube has not yet fused with the subjacent bone.

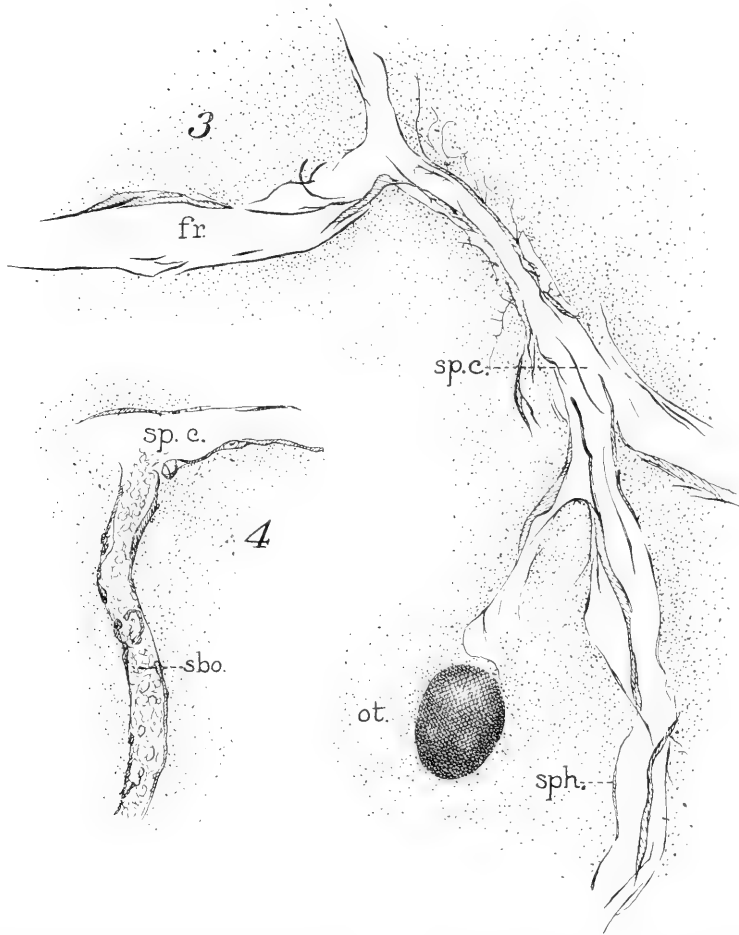


Fig. 3 The condition of the spicules of bone around the supra-orbital canal of larval *Amiurus*, 24.2 mm. in length. The heavy nature of the walls is due to the fact that many spicules are seen superimposed. *fr.*, frontal bone, the ossification of which takes place at the same time as the other bones which are influenced by the canals. The letters *fr* are placed at the midline of the head. *ot.* otolith. *sp. c.*, supra-orbital canal at the point of junction with the suborbital canal leading off to the right. *sph.* the initial deposition of radiating spicules at the point where the sphenotic will later form.

Fig. 4 The suborbital chain of bones on the suborbital canal (*sbo.*) at the region of its junction with the supra-orbital canal in an *Amiurus* larva 50 mm. in length, showing the condition of ossification in the threads and granules. Above the index line leading to the letters *sbo.* the granules are arranged in a ring protecting the tubule opening from the canal at this point.

HISTOLOGY OF THE CANALS AND ASSOCIATED BONES

Kindred ('19) has discussed in such a satisfactory manner the histological relations of the lateral-line canals and associated bones in the early stages of *Amiurus* that little in addition need be given here. His figures are, however, for the most part diagrammatic and fail to show the very intimate relation existing between the spicules of bone, especially in advanced stages,



Fig. 5 Photomicrograph of a lateral-line sense organ in its enclosed canal from the body of *Amiurus*. $\times 450$. This figure shows especially well how far removed and how isolated the sense organs are from the center of osteogenesis which is found on the periphery of the canal (*c. t.*) itself. *c.*, lumen of the canal. *c. t.*, dense connective tissue protecting the contents of the canal and on the surface of which bone deposition begins. *s. o.*, lateral-line sense organ.

fitting very closely to the dense connective-tissue canal with its epithelial lining and mucus cells. In some places the bone seems actually to replace the wall of the canal, as would be expected in the growth of the skull bones. In every phase of their histological structure the canals and the bones bear out the relationship already given. I have not thought fit to follow out the histogenesis of the bone in detail or to try to solve the problem of the highly osteoid nature of fish bone. That phase has little bearing on my problem. Wright ('84, p. 263) has likewise discussed the histological relations of the canals.

SUGGESTED EXPLANATIONS OF THE INFLUENCE OF THE LATERAL-LINE CANALS ON OSTEOGENESIS

The influence of the lateral-line system is suggested by the nature of the ossification around the canals. One explanation which may be offered for this association is that the influence is a mechanical one due to the presence of the tubular canals, which on account of their early appearance, are in a more advanced state of development and the colloidal substance³ on their surfaces earlier attracts the deposition of calcium salts. This influence acting in conjunction with capillary and surface phenomena on the surface of the canals induces the ossification around them. The problem is thus a very complex one and difficult to understand in its final analysis, since the effects of living material on chemical reactions of this nature are little understood.

The spicules, wavy in outline, are laid down parallel to the canals. They look like wavy brown threads. Their irregularity is doubtless due to the irregular disposition around the canals of the osteoblastic cells between which the threads are deposited. The threads are non-crystalline, since they fail to react to polarized light, and their nature is especially evident on the suborbital canal where the threads are shorter (fig. 4). They differ from the otoliths which are crystalline calc-spar.

The deposition of inorganic material in living bodies is of course a very widespread phenomenon, and the nature of the process has been discussed, in a very interesting manner by D'Arcy Thompson ('17). That the deposition differs in different forms of life is evident, as does also the form of the elements. The early formation of osseous material in the so-called membrane bones of the head is especially comparable throughout the range of vertebrates, being very similar in the catfish and the pig.

³ This interpretation has a striking parallel in the field of neuropathology. Recently Doctor Hassin has told me of a case of calcification in a human cerebellum where the calcium gathered in and around the arteries, around whose walls a process known as colloid infiltration had gone on. On the masses of colloid material the calcium was deposited. The arteries represent inactive material, as do the lateral-line canals; the colloidal substance is aggregated around the tubular structures on which the calcium is deposited, and in either case may give rise to bone.

The form of the spicule may depend on its chemical nature, its mineralogical condition and on the influence of the surrounding parts. Bone in the catfish head is laid down in a non-crystalline form because of the slow growth; the early deposition being in the nature of granules, to which other granules are added.

The spicule is directed in its early growth only by the capillary, surface, and chemical influences which prevent it from ranging far from the colloidal bed immediately adjacent to the canals. Later it must respond to the molding influence of the form of the catfish head and to hereditary influences. It is interesting to speculate that the ancient catfish had in its head a series of segmental bones made up of thread-like spicules, like those seen in the young *Amiurus* 24 mm. long, but unfortunately the paleontology of the Siluroids is not sufficiently well known to prove this, the group being unknown prior to the close of the Mesozoic, and the Eocene species differing but little from the modern forms. The part heredity may play is thus impossible of solution. The segmental condition is transitory, having disappeared entirely from the sphenotics in a 50-mm. larva, measured from the tip of the caudal fin.

Another and very important influence exerted by the lateral-line system is that it introduces into the living, active materials of the young head an inactive substance, such as the canals are. The dense connective tissue forming the canal is protective in nature and inactive in function. Actual precipitation of calcium salts takes place usually, not in living, but in either dead or inactive material. This has been the idea advanced by Sims Woodhead ('89), who says that "lime salts, of whatever form, are deposited only in vitally inactive tissue." Another important factor is the degree of lime salts present in the medium in which the animal lives and of the amount of salts taken with the food, since it has been seen that in the presence of excess of lime, the shells of various Foraminifera become greatly altered, strengthened with various excrescences and assuming characters described as proper to other species.

APPLICATION OF EMBRYOLOGICAL DATA TO PALEONTOLOGY

It seems reasonable to state that the only way in which we can explain many paleontological facts is by reference to recent phenomena. We are seeking now to explain why, from the standpoint of development, the lateral-line system in ancient vertebrates is always associated with certain definite osseous elements. A suggested explanation is found in the manner of development of the lateral-line bones in *Amiurus*. It has not seemed necessary to investigate this condition in more than this one form, since from the work of Allis ('04) it is evident that there is a general conformity in the relationship of the bones of the head with certain portions of the lateral-line canals. Hence we are assuming that the phenomenon is constant in the larger aspects.

We have seen that the lateral-line canals develop before the formation of bone. They are precocious structures which carry a mechanical influence into the formation of the skull for the following reasons:

1. They introduce an inactive substance on which calcium salts may be deposited.
2. They attract the early deposition of bone because they furnish a more advanced state of colloidal substances on their surfaces than is found elsewhere in the head except in those regions, such as the occiput, where the deposition of bone is brought about in response to stress.
3. The lateral-line canals, because of their form, furnish a region for the influence of capillary and surface phenomena which are exerted in a purely mechanical way.
4. The deposition of bone is not in response to the nervous stimulus of the sense organs, since bone is not deposited over them until later.

If these explanations seem reasonable, it is possible to further explain our primary problem by stating that we have no reason to doubt that, in the larval stages of the *Labyrinthodonts*, the lateral-line sense organs and canals developed in the same precocious way they do now in modern fishes and that the canals exerted the same mechanical influence on the deposition of bone

then as now. The position of the canals in the ancient larvae of the early amphibians was probably constant and since the canals mechanically determined the formation of bone the relation of the adult elements is constant.

This, of course, explains only the general features of the association, and that is all we are seeking to explain. Why certain isolated segments of bone, as laid down around the larval canals, later unite to form single skull elements is a matter for which we have no uniform explanation. Since the elements of ancient vertebrate skulls differ in their form and arrangements only in details, we must assume that a variety of modifying agencies enter in. The form of the head and hereditary influences are very potent.

Our application of embryological data to paleontology then consists in stating that since in modern fishes the lateral-line canals develop early and exert a mechanical influence on the formation of the peripheral osseous elements, there is no reason to doubt that the ancient amphibians of the Coal Measures, Permian and Triassic possessed in their larval stages similar conditions of an early developed lateral-line system which for the reasons stated exerted a mechanical influence on the deposition of the bones.

SUMMARY

1. The lateral-line sense organs are not trophic for the head skeleton.

2. The association of lateral-line canals with certain definite peripheral osseous elements has been a constant feature in the organization of fishes and Amphibia from Devonian times to the present.

3. The presence of dense connective tissue in the lateral-line canals of the body and of the head is important because it thus furnishes an inactive base for bone deposition.

4. There are two sets of bones in the head of *Amiurus* which are here interpreted as developing in two ways because of: *a*) stress the more powerful influence and, *b*) the mechanical influence of the lateral-line canals.

5. The nature of the influence of the lateral-line canals on the formation of the peripheral osseous elements is found to be mechanical because it furnishes an inactive substance in the form of the dense connective tissue of which the canals are composed. They furnish a more highly organized basis of colloidal material for the attraction of lime deposition on account of their precocious development and because of the mechanical influence of the surface and capillary features present on the surfaces of the canals.

6. The earliest spicules are laid down parallel to the canals. They are wavy in outline and irregular, the irregularity being due to a response to the form of the irregularly placed osteoblasts between which the threads are deposited. The threads are granular and non-crystalline.

7. The suborbital chain of bones, between the segments of which appear the tubules, is not completed until after the 50 mm. stage.

8. The histological features of the canals and associated bones bear out the interpretation given to the appearances in the cleared specimens. This is shown by the intimate association of the spicules of bone to the walls of the canals. The thickness of bone is increased at the expense of the canals, from without inward.

9. The application of these determined embryological facts to paleontology is considered justifiable because of the constancy of structure in the fishes and amphibians. The tubular lateral-line canals are there before bone develops. They are in the way and introduce disturbing factors of a mechanical nature to which the peripheral osseous elements in their formation must respond.

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Resumen por el autor, J. M. D. Olmsted.

Las fibras gustativas y el nervio de la cuerda del tímpano.

Pruebas de que las fibras gustativas de las tres cuartas partes de la región anterior de la lengua pasan desde el nervio lingual a la cuerda del tímpano, en su marcha hacia el cerebro, se encuentran en el hecho de la desaparición de los botones gustativos de un lado de la lengua cuando se secciona la cuerda del mismo lado, mientras que tal cosa no sucede cuando se secciona el nervio mandibular. Las fibras motrices de esta parte de la lengua son transportadas por el nervio mandibular.

Translation by José F. Nonidez
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TASTE FIBERS AND THE CHORDA TYMPANI NERVE

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ONE FIGURE

The presence of taste fibers in the chorda tympani nerve has been demonstrated in two ways: first, by loss of the sense of taste in the anterior part of the tongue (one-third to four-fifths) after injury to or severance of this nerve (Sheldon, '09, for literature to that date; also Sabotky, '18) and, second, there is a single record where sensations of taste, sweet, sour, and bitter, were aroused in a human subject by the direct stimulation of the chorda tympani (Blau, '78).

There is a third method of investigation, the results of which can leave no doubt that taste fibers from the anterior part of the tongue leave the lingual nerve at its junction with the chorda tympani and pass toward the brain through the latter nerve. Vintschgau and Hönigschmied ('76) showed that taste buds disappear from the circumvallate papillae on the tongue of the dog after severing the glossopharyngeal nerve. I have shown that this is also true for the fungiform papillae on the anterior part of the tongue of the dog when the lingual nerve is cut (Olmsted, '21). The taste buds disintegrate and are removed by the phagocytic action of leucocytes within eight days after cutting the nerves. This degeneration of the taste buds is not due to injuries consequent upon operation, since the lingual nerve may be disclosed, separated from the other tissues, and handled in exactly the same manner as in operations where this nerve was cut, and yet no changes will occur in the taste buds. Likewise the cutting of other branches of the lingual nerve which do not pass to the tongue, such as those to the submaxillary gland, is unaccompanied by changes in the taste buds. Hence this disappear-

ance of the taste buds is associated with injury to the nerves connected with these organs.

It is possible, therefore, to follow the course of taste fibers by cutting the nerve tract in which they are supposed to lie and examining the taste buds some eight or more days later. It is hoped that this method, which was suggested to me by Dr. G. H. Parker, will be successful in settling the question of the pathway of taste fibers from the chorda tympani to the brain; this has not been realized as yet.

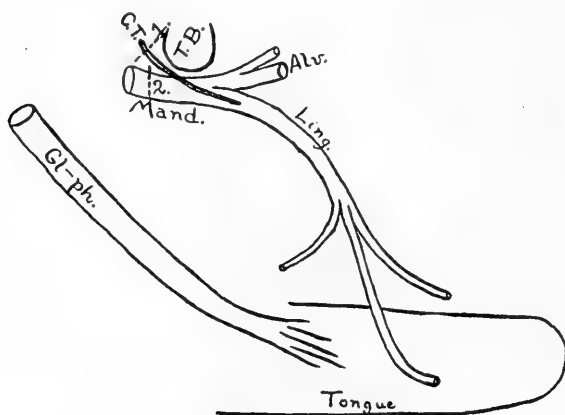


Fig. 1. Diagram showing points at which the nerves were severed. *Alv.*, inferior alveolar nerve; *C. T.*, chorda tympani; *Gl-ph.*, glossopharyngeal nerve; *Ling.*, lingual nerve; *Mand.*, mandibular nerve; *T. B.*, tympanic bulla of the temporal bone; broken line at 1 indicates the position of the cut on the chorda tympani in dogs 12 and 13; broken line 2 indicates the position of the cut on the mandibular nerve in dog 14.

After several unsuccessful attempts I was able to sever the left chorda tympani nerve in two dogs just craniad to its junction with the lingual (fig. 1). There was no injury to the lingual itself and little damage to the surrounding tissue aside from the cutting of the tendinous attachment of the pterygoid muscles. Thirteen days elapsed after the operation on one dog before it was killed and the tongue removed for histological examination, and fourteen days in the second. Fungiform papillae were taken at random from different levels of the anterior three-fourths of the

tongue immediately after the animal's death. These were fixed at once, and sectioned and stained in the usual manner (Olmsted, '21). At the same time the head was dissected. It was found that in both dogs the left chorda tympani had been severed, while the lingual remained unharmed. The results given in table 1 show that all papillae which were examined from the operated side—twelve in one, fourteen in the other—were lacking in taste buds, or the taste buds were in a late stage of degeneration. Papillae from corresponding regions on the unoperated side were normal in every respect.

TABLE 1

DOG	NUMBER PAPILLAE EXAMINED	NUMBER PAPILLAE WITHOUT TASTE BUDS	NUMBER PAPILLAE WITH DEGENERATING TASTE BUDS	NUMBER PAPILLAE WITH NORMAL TASTE BUDS
Left chorda tympani cut				
No. 12	12	11	1	0
No. 13	14	12	2	0
Right side uninjured				
No. 12	6	0	0	6
No. 13	9	0	0	9
Left mandibular nerve cut				
No. 14	14	0	0	14
Operation as extensive as in cutting chorda, but chorda left intact				
No. 6	7	0	0	7

Further proof that the injury of operation was not the cause of the disappearance of the taste buds is afforded by the results of operations on three dogs in which the attempt to cut the chorda tympani was unsuccessful. The incision in all these operations is made just anterior and mediad to the angle of the jaw (following the directions given in figures 238, 239, 240 of Jackson's *Experimental Pharmacology*). After the lingual nerve is disclosed it can be easily traced to the point where it passes between the pterygoid muscles. The tendinous attachments of these muscles must be cut and the tissues separated clear to the tympanic bulla of the temporal bone in order to lay bare the chorda tym-

pani nerve. In dogs with a short deep jaw, the bulldog type, it is almost impossible to disclose the chorda along a sufficient length if damage to the surrounding tissue is to be avoided. But in dogs of the collie type with long shallow jaws the operation is much simpler, especially if the dog is fairly large. Eleven to fourteen days after these three operations the dogs were killed, the tongues removed, and preparations of the papillae made. Six or seven papillae from each dog were examined and all were found to be normal. None had less than two taste buds and the average for each was slightly over four. The three heads were dissected and in all of them both the chorda tympani and lingual nerves on the operated side were intact.

In another dog the left mandibular nerve was cut just cranial to the entrance of the chorda tympani (fig. 1). The dogs with the chorda severed seemed to experience no difficulty in eating or drinking, but were able to use their tongues quite normally. The chorda therefore does not carry efferent fibers to the muscles of the tongue. But this one dog with the mandibular nerve cut had great difficulty, especially in drinking. The left side of the tongue was paralyzed so that the margin could not turn up to form a rim along the edge of the tongue. Consequently, as the animal tried to lap up water from a dish the water ran out the left side of its mouth. The dog was killed on the twelfth day after operation. Dissection showed that the operation was successful for the chorda was intact, and that the mandibular just dorsal to it was severed. Fourteen fungiform papillae were taken at random from different levels of the tongue from the tip to three-fourths of the way back. Every one of them contained at least three normal taste buds and none of them showed signs of degeneration.

Since so many representative papillae from all parts of the anterior three-fourths of the tongue showed no change in their taste buds after cutting the mandibular nerve, while taste buds were absent or in the act of disappearing from papillae taken from similar regions in dogs with the chorda cut, it seems very reasonable to assume that all the taste fibers from this region of the dog's tongue pass to the brain through the chorda tympani and that the lingual carries the motor fibers to the muscles.

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Resumen por el autor, Roy L. Moodie.

Sobre la anatomía endocraneal de algunos mamíferos oligocénicos
y pleistocénicos.

El cerebro de los roedores antiguos no presenta caracteres más primitivos que el de muchos roedores modernos. Desde los tiempos del Oligoceno no ha habido desarrollo cerebral en este grupo. Los insectívoros han experimentado una retrogresión en la estructura cerebral. La prueba de esto se encuentra en el hecho de poseer un neopalio más extenso las formas del Oligoceno, cuando se las compara con las formas modernas. Los carnívoros aeluroides presentan un caso claro de evolución cerebral. Tres macairodontes presentan estados progresivos del desarrollo de la complejidad cerebral. El neopalio era casi tan extenso en los tiempos del Oligoceno como en los mamíferos presentes. El máximo de complejidad cerebral de los gatos fósiles se presenta en *Smilodon*, del Pleistoceno. Dos carnívoros cinoides indican un ejemplo claro de desarrollo cerebral. Este desarrollo está demostrado en la complejidad cada vez más desarrollada del patrón cerebral y en el mayor grado de recubrimiento del cerebro sobre el cerebelo. Los tres tipos de oreodontos representan una ligera cantidad de diversidad entre estos rumiantes de forma semejante a la de un cerdo.

Translation by José F. Nonidez
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ON THE ENDOCRANIAL ANATOMY OF SOME OLIGO- CENE AND PLEISTOCENE MAMMALS

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TWENTY-FIVE FIGURES

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INTRODUCTION

It has been generally assumed by students of comparative neurology that ancient mammalian brains are primitive in nature and that throughout geologic time there has been an evolution of the cerebral cortex. Such an evolution is indicated in the aeluroid and cynoid casts described herewith, this being the first direct comparison of ancient and modern forms to bring out this point.

There have been in my possession for several years a number of endocranial casts of fossil mammals, but I have deferred their description for various reasons. Now that Davidson Black has described ('20) the endocranial casts of *Oreodon* so fully, I feel that there is a basis for further work by those who have not been especially trained in the study of such objects. The field of endocranial anatomy is a very special one and considerable advance has been made since Scott's ('98) results were published. The advancement of knowledge in this field is due largely to the work of G. Elliot Smith, Palmer ('13), and Davidson Black ('15, '20). The change in the conception of the nature of endocranial casts and their faithful reproduction of the surface of the mammalian brain is dated from the actual comparison of natural endocranial casts with the brains and artificial casts by Davidson Black ('15). The impress which the brain makes upon the inner table of the skull varies greatly with the group, and in the casts here described it is found to be most strongly manifested in the Carnivora, reaching its maximum in *Smilodon* from the Pleistocene of California. In a few cases the entire configuration of the cranium is modified by the contained brain, noted especially in *Putorius* by Schwalbe ('04), where the gyri are evident as external elevations on the skull.

It is manifestly impossible for one who has not been especially trained in comparative mammalian neurology to do a great deal in the interpretation of these endocranial casts, but I have been encouraged to place my results on record for other workers in the field. The figures are all accurately done by trained artists and all have been carefully checked with the specimens.

LITERATURE

An important contribution to the study of endocranial casts is that of Tilly Edinger ('21), who has described the natural cast of the brain cavity of *Nothosaurus*, a long-headed reptile of the middle Triassic near Heidelberg. This reptile is interesting in having a huge anteriorly placed pineal opening. Edinger compares the ancient cast with that of the modern alligator and its endocranial cast, calling attention to the fact that the

endocranial cast of *Nothosaurus* only suggests the form of the brain. Similar results have been obtained by other writers on ancient reptilian endocranial casts.

A review of the literature on ancient mammalian endocranial casts will not be made here. All additional literature which has appeared since my annotated bibliography ('15) was printed has been given by Black ('15, '20).

MATERIALS

The nineteen endocranial casts are all from the White River Beds of South Dakota, ranging from Lower to Middle Oligocene. The casts represent the following forms:

1 cast representing a primitive rodent, genus and species uncertain, possible a sciurormorph (figs. 1 and 2). 3 casts representing primitive insectivores, *Ictops acutidens* (figs. 5 to 8).

1 cast representing a primitive saber-toothed cat, *Dinictis felina* Leidy (figs. 3 and 4).

1 cast representing an undetermined felid, possibly *Hopphoneus* (figs. 9 to 11).

1 cast representing a primitive bear-dog, *Daphaenus felinus* Leidy (fig. 12).

8 casts representing the common oreodont, *Oreodon* (*Merycoidodon*) *culbertsoni* Leidy (figs. 22 to 23).

1 cast representing a species of *Merycochoerus*, a large semi-aquatic oreodont, associated with casts of the paranasal sinuses (fig. 17).

1 cast of *Oreodon gracilis* Leidy associated with paranasal sinus casts (fig. 21).

1 cast doubtfully representing *Oreodon gracilis* Leidy (figs. 18 to 20).

1 partial cerebral cast representing the three-toed horse, *Mesohippus* (fig. 24).

I am obliged to Mr. Paul C. Miller, of the University of Chicago, Mr. E. S. Riggs, of Field Museum, and Mr. H. T. Martin, of the University of Kansas, for the loan of material. For aid in confirming the identification of the casts I am indebted to Mr. E. S. Riggs, Dr. W. D. Matthew, and Dr. E. L. Troxell.

The Pleistocene mammals are represented by a partial brain case of *Smilodon* from the Pleistocene deposits of the Rancho la Brea of southern California, and a complete brain case of *Aenocyon dirus*, the giant wolf of the same beds. In order that accurate casts might be made, the task of removing the bitumen and taking correct impressions of the rugose endocranial cavities was entrusted to Mr. Adolph Hammer, director of the plastic studios of the department of anatomy. His success will be evident from the figures of these casts (figs. 13 to 16) made by Miss Genevieve Meakin, artist in the department. Comparisons have been made not only with figures of other casts, but with artificial casts of modern mammals. All the material used in this study will be deposited with the Walker Museum at the University of Chicago.

DESCRIPTION OF ENDOCRANIAL CASTS

The casts are all in fairly good state of preservation, though they have suffered the accidents to which all fossils are subject, being cracked, broken, and weathered. While these accidents have obscured certain of the anatomical features, especially of the olfactory bulb and cerebellum, yet sufficient remains to justify description. Photographs of the casts have not been made, since half-tone reproduction usually destroys the greater part of the detail.

Measurements of each cast have been made on the basis outlined by Black ('20) and his terminology has been adopted. The displacement of the objects had been obtained by immersing them in a large graduate partially filled with water. While this method does not give an accurate conception of the endocranial capacity of each mammal, since the casts are often incomplete, yet the capacity is so closely indicated that it is worth recording.

The fine work of Bolk ('06) has been depended upon for identification of parts of the cerebellum where such was possible and the extremely useful catalogue compiled by G. Elliot Smith ('02) has been the guide in the interpretation of parts of the cerebrum. Particular reference is not made to the many general comparative anatomies consulted, such as Gegenbaur, Sisson,

Reighard and Jennings, Jayne, Wiedersheim, Johnston, and many others. These have proved useful in certain identifications, such as the endocranial blood vessels, nerves, foramina, etc.

Rodentia

A single cast represents this primitive mammalian group. It has not been positively identified, but it has been regarded as "possibly *Paleolagus haydeni* Leidy," which Matthew ('01) refers to as the most abundant hare in the White River beds to which it is limited. A careful comparison of this cast with *Paleolagus* remains in Yale University Museum by Dr. E. L. Troxell has led to the conclusion that it is not an ancient rabbit brain.

The specimen is shown in figures 1 and 2. The cerebrum measures 20 mm. in length; 12 mm. in greatest cerebral width, and the olfactory bulbs, which are beautifully preserved, have a length of 6 mm. and a combined width of 5 mm.

The surface of the brain, as in modern rabbits and in the *Sciuromorpha*, is perfectly smooth. The rugosities which appear in the form of mineral incrustations may represent the meningeal vessels (fig. 1), though I am inclined to think they do not. The smooth projection (fig. 1, *cn*) just anterior to the olfactory bulb is evidently a portion of the cast of the nasal chamber. The olfactory bulbs themselves resemble in great measure those of the modern rabbit. They are smooth, but are shorter and broader than in *Lepus* and in the fossil they are more widely separated. The inferior surface is imperfect, so I am not able to determine the number of *fila olfactoria*.

The only surface marking on the cerebrum is a small sulcus (fig. 2), evidently representing the rhinal fissure. This is placed well down on the lateral surface, so that the neopallium is extensive. The cerebrum is more slender than in the modern rabbit with the pyriform region more protuberant. The lobes are rounded as in the squirrels, not flat as in *Paleolagus* and modern lagomorphs. The cerebellum is imperfectly preserved. The base had been partially destroyed by being mounted on a wire pedestal.

So far as I can learn, the brain cast of no ancient rodent has been previously described.

Troxell ('21), in a recent study of material in the Yale Museum representing *Paleolagus*, with which this cast was compared, remarks that the brain of *Paleolagus* is relatively small and flat, differing from the present cast in length and breadth and in the small size of the foramen magnum. The small size of the brain is merely a substantiation of the law announced by Professor Marsh ('74), which he indicates in these words: "In other groups of mammals, likewise, so far as observed, the size of the brain shows a corresponding increase in the successive subdivisions of the Tertiary."

G. Elliot Smith ('02, p. 195) has called attention to an interpretation of the smoothness of the cortex of the brain of certain rodents which is pertinent here. Contrary to the usual assumption, he believes that the smoothness of the cortex, such as is exhibited by the endocranial cast of the ancient rodent, does not indicate a primitive condition of the cortex for the group. Some of the mammals, such as the hystricomorphine Rodentia, with cerebral hemispheres the same size as the beaver, possess numerous deep sulci. He states: "This is one of the enigmas of cerebral morphology which we are utterly unable to satisfactorily explain at the present time."

Insectivora

Ictops. The three cranial casts discussed under this heading are identified as *Ictops* by Doctor Matthew and Dr. E. L. Troxell and for the sake of convenience I have referred to them under the specific name *Ictops acutidens*, an insectivore of the White River, Oligocene, beds. There is no assurance that the casts represent this species, or even that the casts, found isolated as they were, belong to the same species. There is, however, a wide range of variability exhibited by the skeletal parts of *Ictops*. The family Leptictidae, representing the primitive hedgehogs, is an ancient group of this curious order of mammals, which Doctor Matthew regards as a group "defeated and disappearing in the struggle for existence." In past time the *Insectivora* were of

more importance than now; in fact, they have been considered as representing more nearly than any other living order the primitive central group from which all other mammals have descended. Through the Tertiary the group progressed less than most other orders and several families of them became extinct during that period, while the moles and shrews diverged from nearly similar habits to their present peculiarities, and the hedgehogs, probably, acquired their coat of spines.

All the casts are imperfect, two of them lacking the olfactory region and with imperfect basis cerebri. They are of unequal size with the following measurements:

No. 662

Maximum length.....29 mm.; the bulbus olfactorius is wanting

Maximum width.....22 mm.

No. 628

Maximum length.....30 mm., including the bulbus olfactorius.

Maximum width.....20 mm.

Length of olfactory bulb.....3 mm.

Width of olfactory bulb.....14 mm.

A third cast is somewhat longer with the same cerebral width.

These natural endocranial casts (figs. 5, 6, 7, 8) compare very favorably with the figures of the brain of *Erinaceus europaeus*, the modern hedgehog. They exhibit the same large olfactory bulb, short and broad; the same smooth cerebral surfaces. The cerebellum is partially obscured in all specimens. The only definite indication of a sulcus is a slight groove on the lateral surface, doubtless representing the primitive rhinal fissure (*F.rhin.*) and a slight depression which runs transversely across the anterior end of the cerebrum and doubtless represents an orbital sulcus (*S.orb.*). The position of a lateral sulcus is suggested in one specimen, but it is too imperfect to be definitely identified as such. It is thus evident that the hedgehog brain has not advanced at all in cortical complexity since the Oligocene, and that it had by that time attained all the surface features which the group represents to-day.

A comparison of the present casts with the figure of the brain of the European hedgehog given by G. Elliot Smith ('02, p. 189) reveals a number of important differences in the cerebral surfaces

of these two otherwise closely comparable brains. Judging from the portion of an olfactory bulb remaining on one of the endocranial casts (figs. 7 and 8), the Oligocene hedgehogs were fossorial, since to this underground habit is ascribed the huge olfactory development in the recent form. The olfactory tubercle is not so prominent in the ancient as in the modern form, though its position is well shown in figure 6, where the olfactory trigone may also be discerned. The rhinal fissure on the ancient form is much lower in position, and this rather strikingly increases the neopallial surface in the ancient brain which is so greatly reduced in the modern form. A slight anterior depression, not so strongly marked as in the modern hedgehog, marks the impression known as the sulcus orbitalis. The pyriform lobe in the ancient form is less developed, but its condition is well shown in figure 8.

The basis cerebri is well displayed in one specimen, no. 2762, and reveals some things of importance. The optic chiasma is placed well anteriorly, being only 4 mm. posterior to the base of the olfactory bulb. The olfactory trigone and tubercle are both evident as smooth but not very prominent areas. The bases of the trigeminal nerves are quite large and placed well together. Posterior to these arise the cerebral peduncles which are evident as narrow indistinct bands. The pyriform lobes are small and not protuberant.

As previously stated, the cerebellum in all three specimens of the endocranial casts of *Ictops* is imperfectly preserved. On the posterior end of two of them portions of the cerebellar structures can be discerned. While a description of them is justified, a drawing of them in their imperfect state would be needless. The cerebellum of *Ictops* is simple and possibly primitive, though no more so than the cerebella of some modern mammals, notably *Vespertillio*. At first glance, this part of the brain seems divided into three vertical areas or lobes, but this is an appearance due to the manner of arrangement of the parts of the lobes. The cerebellum, as a whole, is more advanced than the cerebrum, since there are represented three major folds. The lobus anterior is evident in two specimens, but its limits are indistinct, save in one where the sulcus primarius is a prominent depression

separating the lobus anterior from the lobulus simplex. The lobus anterior is wholly without lamellae or other complexities. The lobulus simplex is merely a narrow band running across the posterior surface of the endocranial cast. The lobulus petrosus, the lower part of the pars floccularis, is represented in both specimens. It stands out so sharply as scarcely to be recognizable as a part of the cerebellum. It is a smooth rounded object some 3 mm. in diameter attached by a narrow pedestal to the side of the cerebellum.

Carnivora (Aeluroidea)

Dinictis felina Leidy. The most primitive cat represented in the present assemblage of endocranial casts is *Dinictis felina* Leidy from the Oligocene of South Dakota. The cast (figs. 3 and 4) is an unusually good one, as such objects go, though the olfactory portion is imperfect and the cerebellar portion obscured. The form of the cerebrum is, however, almost perfect and one may see at a glance its typically feline character.

When Scott ('89) discussed the systematic position of *Dinictis* he referred to it as the most primitive cat then known. Later ('13) he maintained this position and referred to the opinion of others that *Dinictis* was in a direct ancestral line with the modern cats. The genus is usually referred to the machairodonts or saber-toothed cats, a line of forms which culminated in the huge Pleistocene species (fig. 16), but was also doubtless close to the stem form from which the modern cats were derived.

The cranium of this light-limbed, cursorial machairodont is somewhat elongated, the upper contour of which slopes sharply downwards and backwards from the highest point of the cranium, just back of the orbits, thus leaving but little room for the brain-case. The face in advance of the orbits is much contracted. On account of these cranial formations, the space allotted the cerebrum is comparatively short, while the cavities of the hind-brain and olfactory lobes are long (Scott, '89). At the time Scott wrote, nothing was known of the form of the brain, save in a general way, and, so far as I am aware, no one has since studied the cerebral form of *Dinictis*.

The brain cast of a related and contemporary form *Hoplophoneus*, a large, heavier-limbed cat, has been briefly and inadequately described and figured by Bruce ('83). The discussion of an endocranial cast which may represent *Hoplophoneus* is given below.

The cerebrum, almost all neopallial in nature, of this primitive cat, *Dinictis*, is quite short and thick (fig. 4), and the simple cerebral pattern is evident and clearly impressed on the indurated material preserved. The lateral sulcus (*S.lat.*) runs almost parallel to the sinus sagittalis, ending gradually and without complexity. At its anterior end this sulcus appears to converge with a slight depression which may represent the coronal sulcus and with the termination of the suprasylvian sulcus (*S.ss.*). The prominence of the endocranial venous sinuses may be correlated with the well-developed sagittal crest which the skull exhibits. The crest is, however, slight compared to its development in *Hoplophoneus*. The suprasylvian sulcus (*S.ss.*) runs approximately parallel to the lateral sulcus, these two being the only clearly marked evidences of cerebral complexity seen on the dorsum of the cerebrum. This gives the cerebrum a remarkably simple aspect, as would be expected from the primitive organization evidenced by the skeleton. Meningeal vessels are not evident and the rhinal fissure is not preserved.

The olfactory region was lost as shown in figure 4, X. The broad broken surface of the olfactory bulb, however, indicates a highly developed sense of smell, for the cats have apparently always been predatory. A comparison of the cerebral features of this cat (fig. 4) with the Pleistocene cat (fig. 16) reveals in a striking manner its evolution.

The form of the cerebellum, as seen from above, is only indicated by the material preserved, since the cast had been badly broken in this place before coming into my hands. The relative size of the cerebellum to the cerebrum and its relation to the broad medulla are worthy of note. There is no indication from the specimen of the cerebrum's bulging over the cerebellum such as is commonly seen in modern carnivores.

The lateral aspect of the natural endocranial cast is especially instructive and is noteworthy on account of the slight development of the cerebral sulci and gyri. A future complexity of pattern is suggested for the descendants of this cat, and is to be seen in *Smilodon* (fig. 16). The sylvian fossa is represented by a widely opened depression, the borders of which merge gradually with the adjacent sulci. The floor of the fossa is perfectly smooth.

Casts of three cranial foramina indicate the positions of only three of the cranial nerves, i.e., the optic, the trigeminal, and the auditory. The optic nerve is placed well forward, the part represented doubtless being subsequent to the chiasma. The trigeminal, as indicated by the canal, was a large nerve, but presents no unusual characters. The acoustic is represented by a partial cast of the internal acoustic foramen and its short canal.

Unfortunately, the basis cerebri is too imperfect for description.

	<i>mm.</i>
Length of entire cast of <i>Dinictis</i>	80
Cerebral length.....	44
Cerebral width.....	40
Gyrus between <i>S. lat.</i> and <i>S. ss.</i> is.....	10

Undetermined carnivore, possibly Hoplophoneus. It is singularly unfortunate that I am unable to positively identify the best-preserved brain cast in my possession, but it was not accompanied by skeletal parts. It is by far the most beautifully preserved cast I have ever seen. I submitted it to Doctor Matthew for an opinion, and he thinks it may be a felid, related to *Hoplophoneus* or *Dinictis*. Since I have already described the brain of *Dinictis* (figs. 3 and 4), the identity of which was rendered certain by accompanying skeletal parts, I conclude that the present cast may be some species of *Hoplophoneus*. This is rendered more probable by comparing the cast with the figures of the brain cast of *Hoplophoneus* given by Bruce ('83), where a close resemblance is seen. Then another interesting fact is that *Hoplophoneus* has a high sagittal crest which in other forms tends to an exaggerated development of the superior sagittal sinus. The present cast (figs. 9 and 10) has such an exaggerated

superior sinus. The skull of *Hoplophoneus* is longer than in *Dinictis* and the brain more dog-like. The arrangement of sulci in the present cast (fig. 10) is almost precisely that figured by Bruce ('83) for *Hoplophoneus*. Bruce's figures, however, show a somewhat more robust brain than the present specimen. In all other respects, save only the absence of a high sagittal sinus in Bruce's figure, the two specimens agree. I am not, however, fully satisfied that the present cast represents *Hoplophoneus*. Its beautiful preservation justifies its description, and we must await future associations of skeletal parts with a good brain cast for a positive determination.

That the cast is not cynoid, and hence does not represent *Daphaenus*, the bear-dog of the Lower Oligocene, is evident in the absence of the sulcus ectolateralis in the present cast (fig. 10). Its felid characters are evident in its robust cerebrum, in the arrangement of the sulci (figs. 9 and 10), and in the base of the brain (fig. 11), especially in the relations of the remnants of the trigeminal nerve.

The endocranial cast of this undetermined felid (figs. 9, 10, and 11) differs markedly from that of *Dinictis* (figs. 3 and 4), especially in its more sharply marked sulci and its more advanced cerebral pattern. It is not so cat-like as *Dinictis*. The cerebrum does not overhang the cerebellum. Dorsally, the lateral sulcus (*S.lat.*) runs without interruption from posterior to anterior end of cerebrum, thus differing considerably from *Dinictis* (fig. 3). The ansate sulcus is not indicated, though it is probably proper to speak of the anterior end of this long lateral sulcus as the coronal sulcus (*S.cor.*). There is no postero-lateral sulcus and the lateral sulcus ends abruptly against a gyrus separating the sulcus from the tentorial depression. The suprasylvian sulcus of *Hoplophoneus*, as figured by Bruce ('83), differs from the present specimen in that Bruce's specimen shows an interruption on the postero-dorsal part of the sulcus. There is no such interruption in the present specimen. The sulcus runs without interruption, clearly and deeply marked (fig. 10), being the more deeply impressed posteriorly. This may be merely a specific difference.

The ectosylvian sulcus (*S.ectosyl.*), absent or only faintly indicated in *Dinictis* (fig. 4), is represented in the present cast by a shallow semicircular groove. It is more clearly indicated on the left than on the right side, although its course is clear in either case. It begins and ends without joining either of the adjacent sulci. The depression labeled sylvian fossa (*F.syl.*) is much narrower and more clearly impressed than in *Dinictis*. It is not, however, so advanced as in *Smilodon* (fig. 16), and *Smilodon* is not so far advanced in this respect as the modern lion. So that we have four stages of development of the sylvian fissure represented. *Dinictis* represents the most primitive (fig. 4). Here the fossa is wide and its floor smooth. The undetermined felid (*Hoplophoneus?*) (fig. 10) represents the next stage of advance followed in sequence by the Pleistocene machairodont, *Smilodon* (fig. 16), and culminating in the lion. The first three forms are doubtless in direct ancestral sequence, but I do not mean to say that the modern lion is derived from the Pleistocene machairodonts. Anteriorly and posteriorly the sylvian depression, in the undetermined felid (fig. 10), is joined by shallow depressions which may represent sulci, the anterior one representing the rhinal fissure, and a faint fissura rhinalis posterior indicating an extensive expansion of the neopallium.

The base of the brain is clearly preserved (fig. 11) and indicates a variety of structures. The olfactory region is broken away (fig. 11, X). The optic nerves (*N.opt.*, fig. 11) are indicated in the bases of the casts of the optic foramina. The optic chiasma is represented as a narrow structure with evidences of a median sulcus. The chiasma is rather longer than in the modern cat. The optic tracts are visible as smooth elevations on either side of the anterior portion of the pituitary body (*Fos.pit.*), which is, of course, represented by the casts of the cavity of the sella turcica. The pituitary is longer and more oval in this ancient cat than in the modern form. Representing as they do diverse lines of the *Aeleuroidea*, we should not expect a close conformity of endocranial structure.

Lying lateral and somewhat posterior to the hypophyseal elevation are three pairs of structures which I have interpreted

as the casts of the foramina orbitale, rotundum and ovale, or those passageways of the three main divisions of the trigeminal nerve, namely, the ophthalmic, the maxillary, and the mandibular. This is strikingly similar to the arrangement in the modern domestic cat. The rounded triangular elevation posterior to the bases of these nerves may be interpreted as the cast of the cavity in which lay the semilunar ganglion, embedded within the dura mater. Separated as the ganglion thus was from the floor of the brain case, it is difficult to interpret the slight ridges running fanwise posteriorly over the surface of the ganglia. They might be fasciculi if seen on the surface of the ganglia themselves. These elevations extend over on to the surfaces of the cerebral peduncles and may be interpreted as indications of small meningeal nerves. The cerebral peduncles are clearly preserved as a V, between the prongs of which is to be seen the well-marked interpeduncular fossa.

The auditory region (*Fos.pet.*) is not readily interpreted. The internal acoustic meatus (*M.a.i.*) is indicated in both sides as a broken oval base. The characters of the medulla are not clearly shown, since the topographic features of this organ are not clearly impressed upon the skull.

The entire cast measures 73 mm. in length and displaces 50 cc. of water. The cerebrum, as preserved, measures 52 mm. in length and 45 mm. in width. The cerebellum extends 36 mm. transversely.

The cerebellum (*cer.*, figs. 9 and 10) is fairly well preserved. This is indeed fortunate, since the region was obscured in *Dinictis* (fig. 3), and we are thus allowed an insight into the nature of the cerebellum in Oligocene cats. The parts are identified according to the terminology of Bolk ('06). As in other primitive mammalian brains, this organ is divided into three main lobules. The lobulus simplex is very prominent, at least I judge this prominence to be that lobule, though I am unable to identify the situation of the sulcus primarius, due to injury to the specimen. A less prominent division lying to either side of the prominent lobulus simplex is the lobulus paramedianus. It lies somewhat further laterally than in the modern lion, but otherwise has

similar relations. Its prominence is due partly to the rather unusual extent anteriorly. The surface of the lobus anterior was a broad, uniformly arched dome similar to the condition found in other cats. On all these subdivisions traces of lamellae are evident, but nowhere are they sharply marked. They are most in evidence on the lobulus paramedianus. A broad foliate lobulus petrosus is also evident on one side, but no details of its structure can be made out.

The preservation of the meningeal vessels in the endocranial cast of the undetermined felid (*Hoplophoneus*?) is worthy of note. The prominence of the superior sagittal sinus has already been mentioned. It rises as a prominent arch posteriorly and evidently sank, anteriorly, between the medial cerebral surfaces. Into the arched portion of this sinus emptied four prominent meningeal veins. The veins are not paired, but served to drain the postero-lateral surfaces of the cerebrum. The postero-lateral dural sinus was robust, extending along the tentorial line. Traces of two prominent arteries are evident. One, corresponding to the middle cerebral artery of the human brain, runs along the floor of the sylvian fissure, bifurcating at the edge of the ectosylvian sulcus. From there spreading indistinctly over the parietal area of the cerebrum, another smaller artery, the middle meningeal, appears to have entered the brain-case through the foramen ovale, sending two prominent rami anteriorly across the areae pyriformes, and another smaller ramus running approximately parallel with the postero-lateral sinus. Minute rami are indicated on the frontal portions of the cast, but their origin is uncertain.

Smilodon. The machairodonts reached their maximum development in the Pleistocene, and I have been fortunate, through the kindness of Mr. E. S. Riggs, in being able to study the left cerebral surface (fig. 16), of a giant saber-tooth cat or tiger, *Smilodon*, from the Pleistocene asphalt of the Rancho la Brea beds near Los Angeles. Scott ('13), in the frontispiece to his book, has given a striking reconstruction of this huge cat. The material at my disposal consists of the left, posterior lateral portion of the brain-case of an adult cat. My interest in the

brain was at once aroused by the very sharply marked inner table of the skull where the sulci and gyri were shown reproduced in a striking pattern. Figure 16 shows the plaster cast made from this incomplete skull, after the asphalt had been carefully removed. The tentorium was damaged, but enough remains to tell something of the nature of the cerebellum. The inner surface of this skull is more sharply marked than in any other mammal which I have seen.

The cerebrum is typically aeluroid and shows a close comparison with the brain of the lion (Elliot Smith, '02, p. 233), the largest modern cat brain I can find figured. Only the posterior two-thirds of the *Smilodon* brain is shown. The gyri are large and prominent and the sulci deep. The lateral sulcus (*S.lat.*) in *Smilodon* has almost precisely the same relations as in the modern lion. The anterior end of this sulcus is lost and the presence or absence of an ansate sulcus impossible to determine. The postero-lateral sulcus is not indicated, but may have been present in the brain. The post-sylvian (*S.postsyl.*) and its continuation, the suprasylvian (*S.ss.*), have almost precisely the same course in *Smilodon* as in the lion, except for the presence of small bifurcations which cannot be determined in the fossil. The posterior ectosylvian sulcus (*S.ectosyl.p.*) is almost exactly the same in the two forms. Only a portion of the anterior ectosylvian sulcus (*S.ectosyl.a.*) is indicated in the ancient cat. A slight depression at the anterior end of the cast is identified as the rhinal fissure (*F.rhin.*). The identification of the sulcus diagonalis (*S.diag.*), in view of the absence of the anterior portion of the cerebrum, is uncertain. The sylvian fissure (*F.syl.*) is more vertical in *Smilodon* than in the lion, and its termination is simpler. If we may be permitted to judge from this portion of the cerebrum which we have preserved, we may say that the cats had attained their cerebral complexity with a surprising degree of completeness by Pleistocene times.

The posterior width of the cerebrum is 68 mm., the estimated length is 75 mm. A single gyrus measures 9 mm. across.

The cerebellar fossa of the incomplete brain case is rather imperfect, so that a careful study of the cerebellum is not possible.

The artificial cast (fig. 16), however, does show something of its form and relations to the cerebrum. The broad widely arched lobus anterior is partly hidden by the cerebrum. Its detailed structure was doubtless partly impressed on the osseous tentorium, which is lost. As compared with *Hoplophoneus* (fig. 9) the lobus anterior is more hidden in *Smilodon*. The prominent projection *Lb.s.* (fig. 16) may indicate the lobulus simplex, though I am unable to find the sulcus primarius. The general shape of the remainder of the cerebellum agrees well with that of the lion (Bolk, '06, fig. 73). A lobulus paramedianus is indicated, but not prominent. This lobule seems to have receded in phylogeny, being more developed in the ancient forms. Doubtless, the entire cerebellum of this huge ancient cat corresponded quite well in most details with that of the modern *Felis leo*. The proportionate size of cerebellum to cerebrum is much greater in *Smilodon* than in the Pleistocene wolf, *Aenocyon dirus*.

Cynoidea

Daphaenus felinus Leidy. The primitive bear-dog, *Daphaenus*, from the Oligocene of South Dakota, is represented (fig. 12) by a weathered cast, the description of which would be unpardonable were it not for the fact that the cerebral complexity and the cerebellar structures are well shown and their simple and primitive nature evident. This cast was figured and partially described (Moodie, '16) in connection with a discussion of the paranasal sinuses of fossil mammals. The terminology there used for the cerebral subdivisions needs revision and a much more adequate figure is given (fig. 12).

Scott ('98) has given a figure of a partially exposed brain cast of this primitive dog from the left side. In order to have a complete account of this interesting brain, I have copied Scott's description:

Very little can be said concerning the brain, since no complete cast of the cranial cavity is available for study. The general shape and development of the brain are, however, indicated in the specimens of *Daphaenus hartshornianus* already described. Its proportions are very different from those found in existing members of the family

(Canidae), a difference which may be briefly stated as largely consisting in the much greater relative size of lobes in the modern species. In *Daphaenus* the brain is narrow and tapers rapidly toward the anterior end; the cerebellum and medulla oblongata are long, the hemispheres narrow and short, and the olfactory lobes very large. The partially exposed cast of the cerebral fossa shows that the cerebral convolutions are fewer, simpler and straighter than in any known species of *Canis* and are even more primitive than those of *Cynodesmus* (figured by Scott in Trans. Amer. Philos. Soc., xvii, 1394, pl. 1, fig. 2). The only sulcus visible in the specimen is apparently the suprasylvian, which is short and pursues a nearly straight course, but curving downward slightly at both ends. From the external character of the skull it is clear that the hemispheres overlap the cerebellum but little.

The dorsal aspect of the present endocranial cast of *Daphaenus* confirms Scott's discussion. The lateral sulcus is almost straight and runs, without complexity, almost parallel with the sagittal sinus. Lying below the lateral sulcus and running almost parallel with it is the ectolateral sulcus which is slightly bent in its course by the broad sylvian fossa, at which point it is joined by the suprasylvian sulcus. On account of weathering, these are the only features observable on this cerebral cast. Length of entire cast is 74 mm., length of cerebrum is 52 mm., width of cerebrum is 43 mm.

The cerebrum of *Daphaenus* overhangs the cerebellum to some extent, but to a much less degree than in *Aenocyon* (fig. 14) or any of the modern cynoid Carnivora.

The lobules of the cerebellum (fig. 12) are clearly indicated and these have been identified according to the terminology proposed by Bolk ('06), following the suggestion of Black for the adoption of a uniform terminology. The eroded condition of the specimen prevents the accurate determination of many minor features, but the lobus anterior is not hidden, except a slight anterior portion, by the cerebrum. In fact, a comparison of the cerebella of *Daphaenus* and *Aenocyon* shows what an enormous advance the Pleistocene form has undergone. The sulcus primarius is indicated by a depression, but its identity is uncertain. The lobulus simplex is hard to differentiate from the lobus posterior. A lobulus paramedianus is prominent, but without details of structure. The condition of this lobule is

similar to its condition in *Hoplophoneus* (?) (fig. 9) in that it lies so far removed from the median line. An indefinite lateral projection doubtless indicates a lobulus petrosus, but of its form and structure nothing can be told. The size of the cerebellum as compared to the cerebrum is much larger in *Daphaenus* than in *Aenocyon*.

Aenocyon (*Canis*) *dirus* Leidy. The giant wolf (*Aenocyon dirus* Leidy), found so abundantly in the asphalt deposits of southern California, was and is the largest representative of its kind. The skeletal remains of this form are very abundant in the Rancho la Brea beds and every detail of its skeletal organization has been discussed by Merriam ('12). The skull is unusually large and the brain-case capacious, the inner table of which is strongly marked by the gyri and sulci and by the meningeal vessels (figs. 13, 14, and 15). The artificial cast from which these figures were made was reproduced from a single practically complete brain-case of this wolf, given the writer by Mr. E. S. Riggs. The brain-case was carefully sawn, longitudinally, and the hard-packed asphalt matrix removed until the inner surface was clean. In this process the delicate osseous tentorium was partially destroyed. It would be important to have a series of casts of the endocranial cavities of various individuals of this wolf—a task readily accomplished by those having access to a number of perfect skulls. I have contented myself with a study of this single cast. A series of such casts of the brain of this wolf has been made by Dr. J. C. Merriam, and a discussion of them will be included in his Rancho la Brea studies. The robust brain of this giant wolf may be readily compared with the modern dog. A comparison was made with an endocranial cast from the skull of a large bulldog, the most obvious differences being the more sharply marked sulci in the extinct form. If complexity of cerebral pattern is any indication of intelligence, then the Pleistocene wolf was a very intelligent creature. The increase in complexity shown by this wolf (figs. 13, 14 and 15) will be more striking if one compares it with the simple pattern of its Oligocene forebear, *Daphaenus* (fig. 12).

The cerebrum of *Aenocyon* (*Canis*) *dirus* almost obscures the cerebellum from a lateral view, so greatly does the neopallium overlap the hindbrain. The cerebellum in this ancient wolf actually lies in a cavern excavated in the posterior wall of the cerebrum and is only evident from a posterior aspect. The olfactory bulb in *Aenocyon* is more straight and does not bend so sharply downwards as it does in modern dogs.

The lateral sulci (*S.lat.*) of the two hemispheres (fig. 13) are unequal in their development and somewhat asymmetric, the right sulcus being farther away from the median border of the hemisphere than the left. Posteriorly, the sulci are associated in their origin with the postero-lateral sulcus (*S.postlat.*, figs. 13 and 14). Anteriorly, the lateral sulci are related to the coronal (*S.cor.*) and ansate (*S.ans.*) sulci (fig. 14), though these last-mentioned sulci do not represent direct continuations of the lateral sulcus as in many modern *Cynoidea*, but are related to it by a shallow depression connecting the three. The gyrus between the lateral sulcus and the median surface of the cerebrum is not a continuous ridge, but is interrupted by transverse depressions, giving the gyrus a bumpy appearance. The sagittal sinus is less developed, proportionately, in *Aenocyon* than in *Daphaenus*. This decrease in size is to be correlated with the growth of the skull and decrease in size of the sagittal crest and the growth of the neopallium.

The postero-lateral sulcus, bilaterally symmetrical, is almost a direct continuation of the lateral, the only interruption being a broad, shallow depression (fig. 14). Terminal rami of the *arteria meningeae media*, clearly evident on the right side of the cast, cross the posterior end of the lateral sulcus and the postero-lateral sulcus. The ectolateral sulcus, equal on the two sides, is sharply marked and its arc is parallel dorsally with the lateral sulcus and posteriorly with the postero-lateral sulcus. The suprasylvian and postsylvian forms a similar arc within the ectolateral. At the upper end of the sylvian depression three trunks of the middle meningeal artery are evident. The orbital sulcus (*S.orb.*) is represented by a short depression near the olfactory bulb, and it is continued downward posterior to the

olfactory trigone until it disappears in the broad sylvian depression (fig. 14). The rhinal fissure is suggested by a shallow depression inferior and parallel to the posterior limb of the orbital sulcus.

This brain on the whole is even more advanced in its cerebral complexity than many of the modern Cynoidea. The gyri are more complex, with more frequent modifications than is seen even in the domestic dog. Perhaps the cerebral complexity of a wild animal is to be correlated with its incessant necessity for self-preservation—an instinct more or less dormant in domestic animals. It is also possibly true that there is no adequate explanation of such cerebral complexity. This being exhibited in one branch of the rodents and absent in another group would indicate something of the puzzling nature of this problem.

The base of the brain is well shown in figure 15, and its features are so clearly represented there that a detailed explanation of its anatomy will not be given. It is, of course, understood that the elevations labeled as cranial nerves are casts of the foramina through which the nerves coursed. The pituitary elevation is seen posterior to the internal carotid artery.

The cerebellar fossa of *Aenocyon* is not so clearly marked as is the cerebral, hence a cast of the fossa does not give quite so good an idea of the endocranial anatomy as one would desire. The form of the lobus anterior is partly hidden by the overhanging cerebrum, and I am unable to locate the sulcus primarius. There is a prominent portion which I take to represent the lobulus simplex. The lobulus paramedianus lies close to the lobus posterior, much as in the modern dog, and it is about as slender. There is a well-developed lobulus petrosus and all subdivisions have indications of lamellae, thus indicating a modern cynoid development in this region.

The artificial endocranial cast of *Aenocyon dirus* has the following measurements:

	mm.
Length of entire cast as figured.....	95
Greatest length of cerebrum.....	70
Greatest width of cerebrum.....	65
Length of olfactory bulb.....	15
Width of olfactory bulb.....	16

The endocranial capacity of this ancient wolf is suggested by the fact that the endocranial cast displaces 120 cc. of water.

Endocranial blood vessels. The surface of the brain of *Aenocyon* is richly supplied with blood vessels, of which an interpretation is here given. The middle meningeal arises as a sharply marked artery, entering the skull through the foramen ovale. Immediately after its entrance into the endocranial cavity, it lies in a groove so deep and sharply cut as almost to be a canal. Coursing posteriorly, it emerges on the lateral surface of the brain at the lower level of the postero-lateral sulcus. The canal in which the artery runs is so sharp, as if it might have been cut on the inner table of the skull with a graver's tool. As the artery approaches the ectosylvian sulcus, coursing anteriorly, it divides into three rami. The posterior arises at right angles to the main artery and supplies the surface posterior to the ectolateral sulcus. The middle ramus runs also posteriorly, but supplies the upper parietal areas. The anterior ramus, interrupted in its direct course by the rami of the mid-cerebral artery, courses obliquely anterior, and supplies the anterior surfaces of the parietal areas and the posterior areas of the frontal region, where its minute subdivisions are interspersed between the ramifications of the anterior meningeal artery. The middle meningeal is thus the most important of all the arteries of the dura mater—more important even than in the human, in that it supplies a greater area.

The anterior meningeal is not so readily followed, but it first appears alongside of the olfactory bulbs, sending many minute rami across the anterior dorsal region.

A ramus of the occipital artery makes a rather well-marked groove in the cerebellar fossa, running almost parallel with the infero-lateral dural sinus.

In the sylvian fossa is found the cast of a very large artery which is by no means as sharply marked as the middle meningeal. This artery I regard as the mid-cerebral artery, its indistinct canal being due to the interference of the dura mater. Its stem first appears as a well-rounded vessel between the optic chiasma and the root of the trigeminal nerve. Coursing at first slightly

anterior, it rounds the pyriform lobe and appears on the lateral surface in the floor of the sylvian fossa. Running within the sylvian fissure, it meets the middle meningeal rami near the ectosylvian sulcus, and from that point the course of the artery is obscure.

The venous dural sinuses are not particularly worthy of note, since they present no unusual features; but emptying into the superior sagittal sinus are cerebral veins. A pair of these of unusual size and prominence is shown in figure 13. These lie just posterior to the ansate sulcus.

Artiodactyla

Oreodon (Merycoidodon) culbertsoni Leidy. Since Davidson Black ('20) has described so fully the endocranial anatomy of *Oreodon*, with a reconstruction of the brain, little further need be added here concerning the general nature of this interesting pig-like ruminant. I have, however, shown in figures 22 and 23 the dorsal and ventral views of a well-preserved cast of this animal, which is rather unusual in showing very nicely the modeling of the cerebrum, both above and below. In general the sulci are similar to those described by Black ('20) and their condition is shown in figure 22. This brain shows an unusual development of the dural sinuses, the sagittal (*Sin.sag.*) and the infero-lateral (*Sin.i.lat.*). This cast is further unusual in presenting the olfactory bulbs, a portion which is often lost in endocranial casts of this animal. Length of cerebrum, 40 mm.; width, 38 mm.; width of olfactory bulb, 5 mm.

On the base the tuberculum olfactorium is rounded and well marked. The pituitary elevation is evident as an elongated elevated mound between the large rounded areae pyriformes. Save for the general shape of the cerebellum, nothing can be told of this region. This cast displaces 41 cc. of water.

Oreodon gracilis Leidy. Another cranial cast doubtfully assigned to *Oreodon gracilis* is shown in figures 18, 19, and 20. The cast is small when compared to another cast of the same species, but the sulci are well developed. The entire endocranial cast measures 60 mm. in length. The cerebrum is 36 mm. long

and 32 mm. across its widest part. It displaces 20 cc. of water. The cerebral pattern is so similar to that of *Oreodon*, as figured by Black ('20), that nothing need be said concerning it, save to point out the absence of all complexities. This, however, may be due to the cast's being that of a young animal in which the complexities had not yet impressed themselves on the inner table of the skull, or it may be a specific character.

The cerebellum shows the same prominence as indicated by Black in the specimens he studied, but otherwise the cast is too eroded to add to our knowledge of this important phase of endocranial anatomy.

Another larger cast of *Oreodon gracilis* and apparently of a more mature individual has recently been loaned me by Mr. H. T. Martin. This cast, likewise shows no complexities in the sulci of the cerebrum. The cast is broken at the tentorial line, but anteriorly there is a magnificent exhibition of the paranasal sinuses, especially interesting in showing the relation of the olfactory bulbs to the frontal sinuses (fig. 21). The length of the cerebrum measured from the tip of the olfactory lobe is 48 mm., the width is 35 mm. The outer ends of the olfactory bulbs are flattened obliquely against the ala ossis frontalis, thus causing the bulbs to end in median sharp points. The upper surfaces of the bulbs are smooth and rounded with a cleft 4 mm. wide and 6 mm. long separating the anterior parts. They join the cerebrum by a common base. This particular region was badly broken in the specimen of *Merycochoerus*.

The casts of the paranasal sinuses, while not so complete as those previously figured (Moodie, '16), are of especial importance in showing the relations of the sinus frontalis and the various sacculations of the sinus maxillaris superior to the olfactory bulb. The casts of the frontal sinuses are mostly broken, but the portions remaining indicate a multiple nature for this sinus in *Oreodon gracilis*, as it is found to be in *Merycochoerus*. Three small sacculations of the sinus maxillaris superior come into intimate relation with the olfactory bulb, being separated from actual contact by the paper-like alae of the frontal bone. The smooth surfaces of all the casts of the sinus maxillaris superior,

of which there are five on each side, are covered with a rich network of rami of the arteria ethmoidalis supplying the mucosa sinus paranasali. Judging from the condition in *Merycochoerus*, the paired median sacculations of the sinus frontalis lay immediately superior to the olfactory bulbs, and we have these bulbs exposed in *Oreodon gracilis* because the sinuses have been broken away.

Merycochoerus. In his discussion of the literature on endocranial casts Black ('20) called attention to errors in a previous figure of this endocranial cast (fig. 17) which was briefly referred to (Moodie, '16) in conjunction with a discussion of the preservation of casts of the paranasal sinuses. The terminology has been revised in accordance with the suggestions of Black ('20) and a new figure showing more carefully the modeling of the brain is given (fig. 17). The sulci and other cerebral markings are very similar to those of *Oreodon*. The dural venous sinuses are likewise fully as prominent. The comparative sizes of the cerebrum and cerebellum are the same in the two forms. Since *Merycochoerus* is a larger animal than *Oreodon*, the cerebrum is correspondingly larger, measuring 50 mm. in length by 48 mm. in greatest width. This cast displaces 77 cc. of water.

Mesohippus. An incomplete cast from South Dakota (fig. 24) represents this form. Cope has figured the brain cast of a related early horse, *Merychippus isonesus* from the Deep River beds of Montana. These figures with many others were issued in a quarto volume under the supervision of Dr. W. D. Matthew ('15) by the American Museum of Natural History. The brain cast is poorly figured, and since it is readily accessible, being no. 8105 of the Cope Collection in the American Museum, should be restudied with additional material. The incomplete cast referred to *Mesohippus* (fig. 24) is in the collections of the University of Kansas. Since the endocranial anatomy of the early horses has been incompletely studied, this material will form a basis for such a study.

Osborn ('88) has described the brain cast of *Mesohippus*, and Scott ('91) has discussed the brain case of this genus, comparing it to the modern horse. Since the present cast (fig. 24) adds

nothing of importance to Osborn's ('88, p. 87) description, his words, copied herewith, will apply.

Mesohippus had a large and well-convoluted brain. The length and breadth indicate that it weighed about one-third as much as the brain of the recent horse, while if we estimate the body weights of the fossil and recent animals by the relative size of the humeri, the brain of the Miocene species was proportionally heavier. The cerebrum of the horse is, however, much more highly convoluted, and the frontal lobes are relatively broader. The Mesohippus brain is distinguished in a marked manner by the longitudinal direction of the parietal and occipital sulci, and by the transverse frontal sulci, from the oblique sulci of all recent ungulates. In fact, in this respect it bears a marked general resemblance to the brain type of recent Carnivora, and conforms with the higher Ungulata of the Eocene. To this may be added that the hemispheres are narrower and less capacious in the fossil, and as in all the lower members of the ungulate series, they taper much anteriorly. This brain shows in the parietal and occipital region very close agreement with the principal fissures of the equine brain as figured by Krueg, but in the frontal region the agreement is much less close, owing to the transverse direction of the frontal sulci.

The high degree of cerebral development indicated by the cast of Mesohippus (fig. 24), by Osborn's ('88) discussion and figures of the endocranial cast, by Scott's ('91) description of the brain case of Mesohippus, and by Cope's (see Matthew '15) figures of the brain cast of Merychippus is strikingly at variance with the other Oligocene Mammalia studied. Mesohippus, an Oligocene tridactyl horse, exhibits in its cerebral pattern a much closer approach to the brain of the modern horse than the brain of Daphaenus, for instance, does to the cerebral pattern of the modern Canidae. This is an interesting example of accelerated development of cerebral pattern in the equine group. The brain of the Oligocene horse had already attained a high degree of complexity and, while still somewhat primitive, it is remarkably modern in its development.

SUMMARY

The brain of the ancient rodents, as indicated in the single cast studied, shows no more primitive characters than many modern rodents. There has been no cerebral development in this group since Oligocene times.

The Insectivora, as indicated by *Ictops*, have retrograded in cerebral structure, as they have in other ways. This is shown by the fact that the Oligocene forms had a much more expansive neopallium than modern forms, as in the European hedgehog. There has been no advance in cerebral complexity in the group since Oligocene times.

The aeluroid carnivores studied show a clear case of cerebral evolution. The three machairodonts, *Dinictis*, *Hoplophoneus* (?), and *Smilodon*, show progressive stages in the development of cerebral complexity. The neopallium, in this group of cats, was almost as extensive in Oligocene times as now. The rhinal fissure occupies nearly the same position in all forms studied. The acme of cerebral complexity of the fossil cats studied was reached in *Smilodon* from the Pleistocene, though a somewhat greater complexity is indicated in some modern cats. Modern cats, however, are not descended from these machairodonts.

The two cynoid carnivores, *Daphaenus* and *Aenocyon*, indicate another clear instance of cerebral development since Oligocene times. This development is shown in the developing complexity of cerebral pattern and in the greater overhang of the cerebrum over the cerebellum. A close study of the meningeal arteries in the Pleistocene wolf reveals important endocranial characters.

The three types of oreodonts shown represent only a slight amount of diversity among these pig-like ruminants.

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FIGURES

ABBREVIATIONS

<i>A. car. int.</i> , internal carotid artery	<i>N. oph.</i> , ophthalmic nerve
<i>A. cer. med.</i> , mid-cerebral artery	<i>N. tri.</i> , trigeminal nerve
<i>A. men. ant.</i> , anterior meningeal artery	<i>S. ans.</i> , sulcus ansatus
<i>A. men. med.</i> , medial meningeal artery	<i>S. cor.</i> , coronary sulcus
<i>Ar. pyr.</i> , pyriform area	<i>S. cruc.</i> , cruciate sulcus
<i>bo.</i> , olfactory bulb	<i>S. diag.</i> , diagonal sulcus
<i>cer.</i> , cerebellum	<i>S. ectol.</i> , ectolateral sulcus
<i>cn.</i> , nasal cavity	<i>S. ectosyl.</i> , ectosylvian sulcus
<i>Crb.</i> , cerebrum	<i>S. ectosyl. a.</i> , anterior ectosylvian sulcus
<i>F. con.</i> , precondylar foramen	<i>S. ectosyl. p.</i> , posterior ectosylvian sulcus
<i>F. rhin.</i> , rhinal fissure	<i>S. lat.</i> , lateral sulcus
<i>F. syl.</i> , sylvian fissure or fossa	<i>S. n.</i> , nasal septum
<i>For. lac. ant.</i> , foramen lacerum anterior	<i>S. orb.</i> , orbital sulcus
<i>For. ov.</i> , Foramen ovale	<i>S. post. lat.</i> , postero-lateral sulcus
<i>Fos. pet.</i> , petrosal fossa for lodgement of temporal bone	<i>S. postsyl.</i> , postsylvian sulcus
<i>Fos. pit.</i> , pituitary fossa	<i>S. pseudo.</i> , pseudosylvian sulcus
<i>Lob. pet.</i> , lobulus petrosus	<i>S. ss.</i> , suprasylvian sulcus
<i>Lb. s.</i> , lobulus simplex of cerebellum	<i>Sin. f.</i> , frontal air sinus
<i>M. a. i.</i> , internal auditory meatus	<i>Sin. i. lat.</i> , inferolateral blood sinus
<i>Med.</i> , medulla oblongata	<i>Sin. max.</i> , maxillary air sinus
<i>N. ac.</i> , acoustic nerve	<i>Sin. max. sup.</i> , Superior maxillary air sinus
<i>N. fac.</i> , facial nerve	<i>Sin. sag.</i> , superior sagittal blood sinus
<i>N. man.</i> , mandibular nerve	<i>X</i> , broken surfaces
<i>N. max.</i> , maxillary nerve	<i>T. o.</i> , Tuberculum olfactorium
<i>N. ocm.</i> , oculomotor nerve	<i>V. cer.</i> , cerebral veins
<i>N. op.</i> , <i>N. opt.</i> , optic nerve	

Fig. 1 Dorsal view of the endocranial cast of a primitive rodent, possibly a sciutomorph. The cast displaces 2 cc. of water. White River Oligocene of South Dakota. No. 629 Walker Museum, University of Chicago. The subdivisions of the brain are perfectly obvious. The entire length of the cast is 24 mm.

Fig. 2 Right lateral view of the same cast.

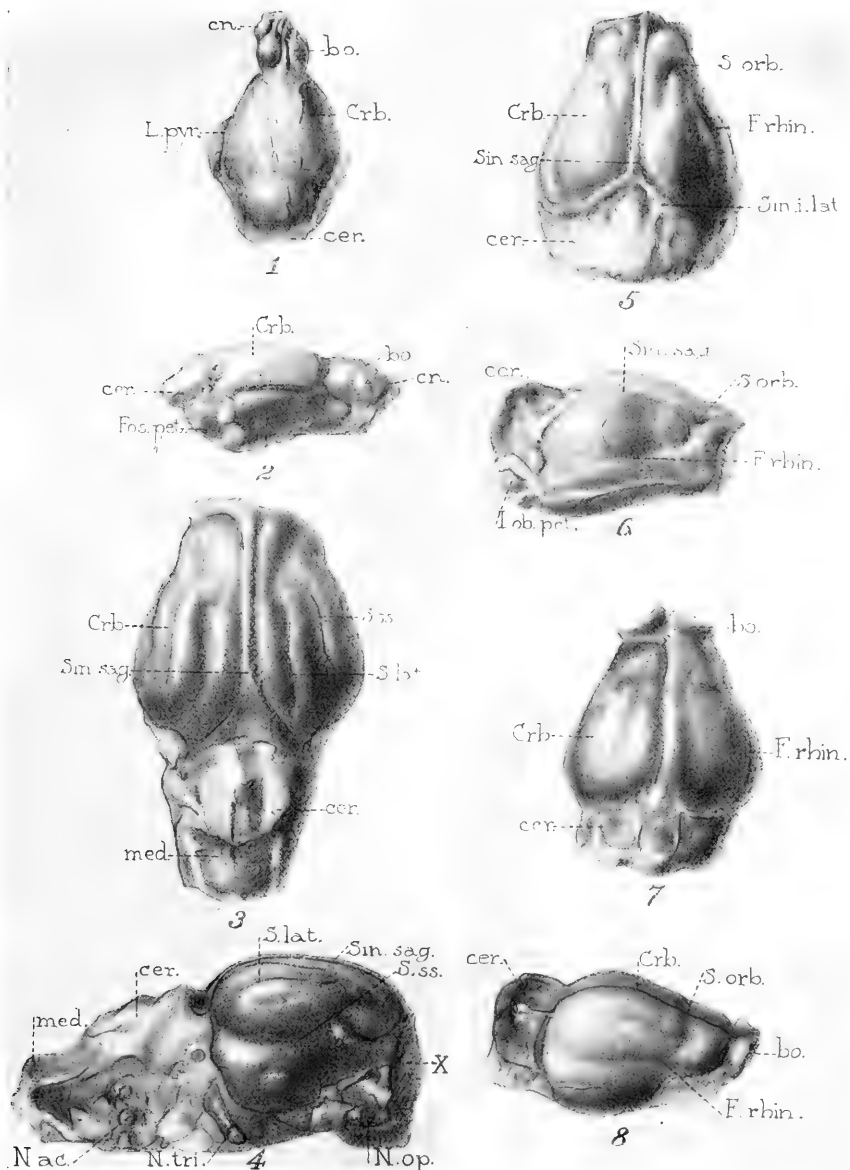
Fig. 3 Dorsal view of the endocranial cast of a primitive saber-toothed cat, *Dinictis felina* Leidy. White River Oligocene of South Dakota. The cast displaces 52 cc. of water. Field Museum. The entire length of the cast as figured is 80 mm.

Fig. 4 Right lateral view of same cast.

Fig. 5 Dorsal view of endocranial cast of a primitive insectivore, *Ictops acutidens*, of the family Leptictidae. The cast displaces 5 cc. of water. No. 622, Walker Museum, University of Chicago. White River Oligocene. Length of entire cast, 27 mm.

Fig. 6 Right lateral view of same cast.

Fig. 7 and 8 Dorsal and right lateral views of same genus, possibly the same species. No. 628, Walker Museum, University of Chicago. Since this cast displaces only 4 cc. of water, it is smaller than the cast in figures 5 and 6. Length of entire cast, 28 mm.



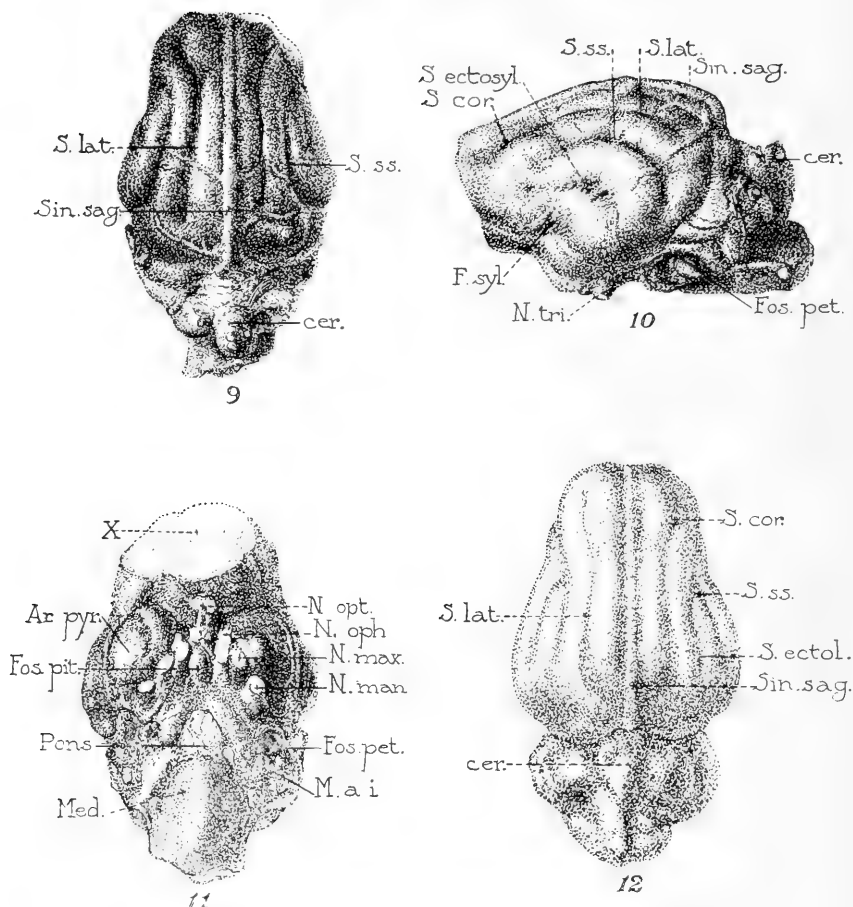


Fig. 9 Undetermined felid, possibly *Hoplophoneus*, a machairodont of the Lower and Middle Oligocene. White River beds of South Dakota, a large contemporary of *Dinictis*. The olfactory region is broken away. The entire cast has a length of 73 mm. and displaces 50 cc. of water.

Fig. 10 Left lateral view of same endocranial cast, showing relation of cerebrum to cerebellum, the course of the sulci and the cast of the precondyloid foramen, running along the medulla.

Fig. 11 Base of same endocranial cast, showing position of cranial nerves as indicated by the casts of the cranial foramina transmitting those nerves. I have regarded these casts as nerves and they are so labeled. *N. opt.* indicates the position of the optic nerve, but is in reality the cast of the foramen opticum. Similarly, *N. oph.* = foramen orbitale; *N. max.* = foramen rotundum, and *N. man.* = foramen ovale.

Fig. 12 Dorsal view of the endocranial cast of *Daphaenus felinus* Leidy, a bear-dog from the Lower Oligocene beds of South Dakota. The cast was somewhat weathered, but the sulci are still evident. The ventral surface is entirely eroded. Length of entire endocranial cast, 74 mm.

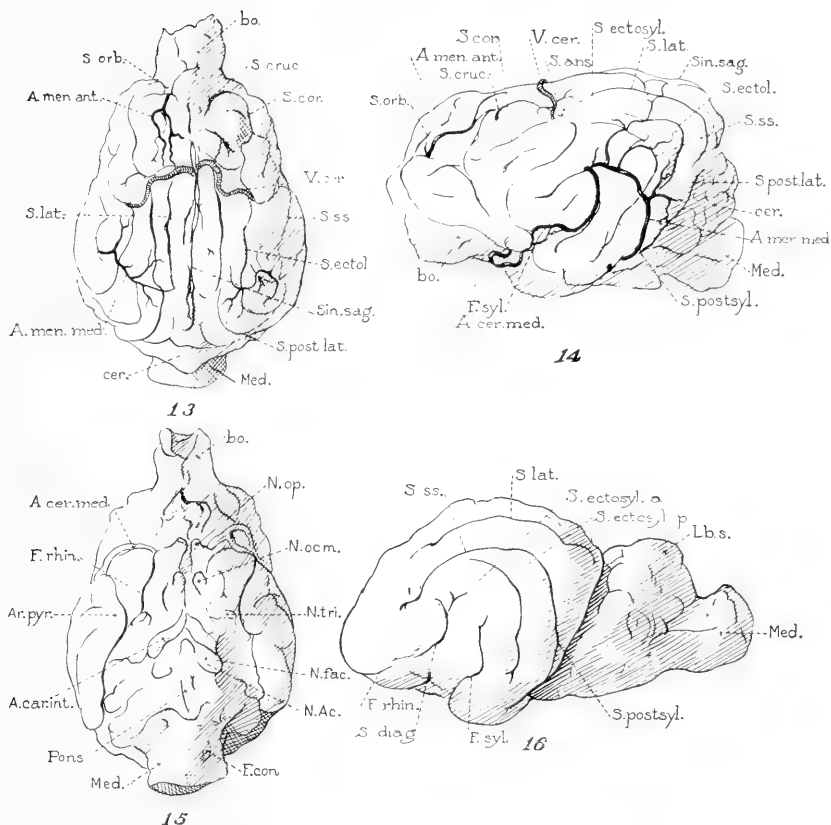


Fig. 13 Dorsal aspect of the artificial endocranial cast of *Aenocyon* (*Canis*) *dirus* Leidy from the Pleistocene of the Rancho la Brea beds of southern California. It will be noted how the overlapping neopallium almost obscures the cerebellum. See list of abbreviations for terminology. The length of the entire cast is 96 mm.

Fig. 14 Left lateral view of same cast. The right side shows the middle meningeal arteries more clearly.

Fig. 15 Base of same cast.

Fig. 16 Left lateral aspect of the portion of brain, as seen in an artificial cast, of the huge saber-toothed cat, *Smilodon*, from the Pleistocene of the Rancho la Brea beds of southern California. Only the posterior two-thirds of the cerebrum is shown. The wide space between the cerebrum and cerebellum is exaggerated, due to the difficulty of casting around the rugose osseous tentorium.

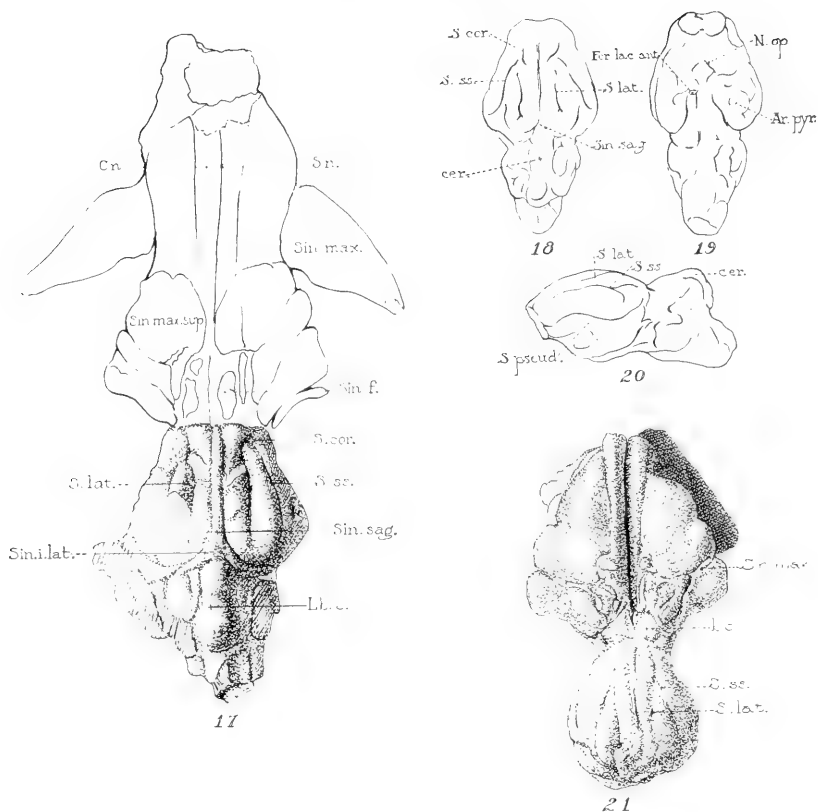


Fig. 17 Endocranial cast with casts of nasal cavity (*C. n.*) and paranasal sinuses (*Sin. max.*, *Sin. f.*, *Sin. max. sup.*) of a large semi-aquatic oreodont, *Merycochoerus*, from the White River Oligocene. The entire brain cast measures 80 mm. in length.

Fig. 18 Dorsal view of an endocranial cast of a small oreodont, possibly *Oreodon gracilis* Leidy, from the Lower Oligocene of South Dakota. The entire endocranial cast measures 60 mm. in length and displaces 20 cc. of water.

Fig. 19 Base of same cast.

Fig. 20 Left lateral view of same endocranial cast. The long sulcus (*S. pseud.*) is correlated with the same sulcus in *Oreodon culbertsoni* Leidy, as figured by Black ('20, fig. 23).

Fig. 21 Relation of cerebral cast to paranasal sinuses in *Oreodon gracilis* Leidy. The entire cast as figured, measures 98 mm. in length; the cerebrum has a length of 40 mm. from the base of the olfactory bulbs. It will be noted that the form of the cerebrum is more robust in this specimen of *Oreodon gracilis* than in the one shown in figures 18, 19, and 20. The cerebral pattern is the same in the two forms, though the sulci are more deeply cut in the smaller cast.

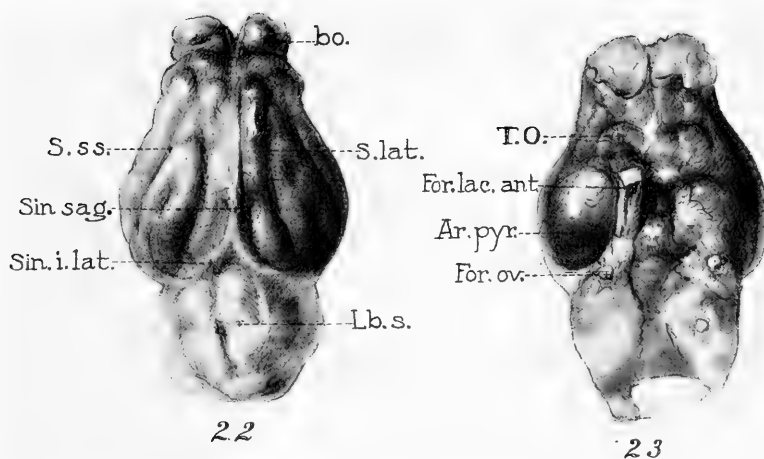
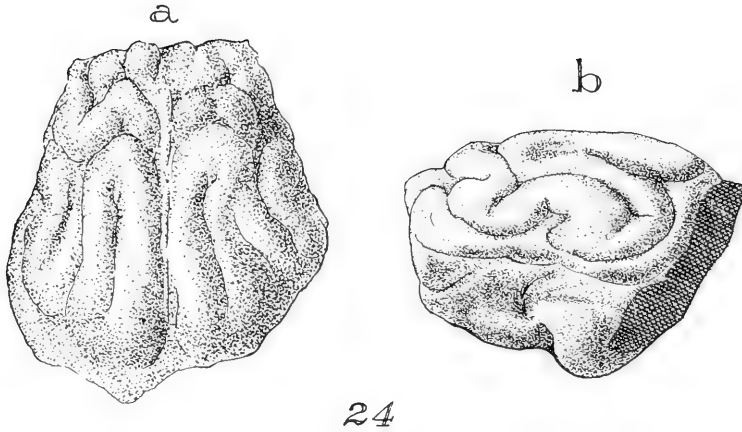


Fig. 22 Dorsal view of endocranial cast of *Oreodon culbertsoni* from the Oligocene of South Dakota, showing the modeling of the cerebrum. The entire cast measures 67 mm. It displaces 41 cc. of water. See list of abbreviations for the meaning of labels.

Fig. 23 Ventral view of same brain cast.



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Fig. 24 Partial endocranial cast identified as representing the cerebrum of *Meshippus*, the early Tertiary three-toed horse. a, Dorsal view of cast; b, Left lateral view of same cast.

The fundamental equine cerebral pattern is already laid down in this early Tertiary horse, from which the cerebrum of *Equus* differs only in size and complexity of pattern. Length of cast, 60 mm.

Fig. 25 Comparative sizes of the ancient mammalian endocranial casts described. Projection drawings representing cross-sections through the widest parts of the cerebrum, made by viewing the casts from directly in front. All two-thirds natural size.

- a, Oligocene rodent, uncertain.
- b. Small specimen of *Ictops*, Oligocene hedgehog.
- c, Larger specimen of *Ictops*, Oligocene hedgehog.
- d, *Dinictis*, primitive Oligocene cat.
- e, *Hoplophoneus* (?), Oligocene machairodont.
- f, *Smilodon* (reconstructed), giant saber-tooth cat.
- g, *Daphaenus*, Oligocene bear-dog.
- h, *Aenocyon*, giant Pleistocene wolf.
- i, *Oreodon*, the common Oligocene pig-like ruminant.
- j, *Oreodon gracilis* Leidy.
- k, *Meshippus*, the three-toed horse (reconstructed). The prominence of the temporal lobes is due to the imperfect nature of the cast.



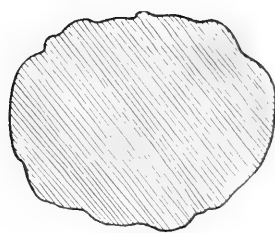
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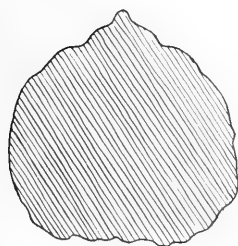
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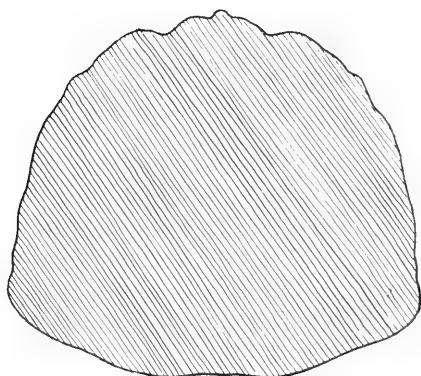
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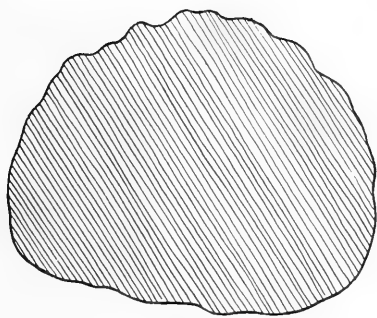
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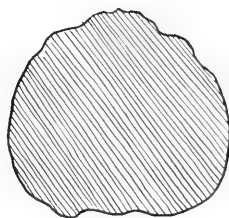
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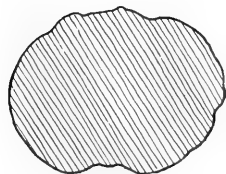
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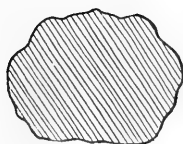
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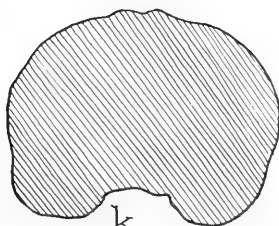
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Resumen por el autor, William A. Hilton.

El sistema nervioso de los Phoronida.

El autor ha llevado a cabo experimentos y observaciones sobre dos especies de Phoronida. En estas especies el sistema nervioso central está solamente separado parcialmente del epitelio superficial. En la porción central del sistema nervioso las fibras y células son claramente más abundantes. En conexión íntima con este sistema nervioso central existen los órganos lofoforales, bien desarrollados. En conexión con el sistema nervioso central ha hallado el autor un cordón longitudinal izquierdo. Si no es una nueva estructura puede ser algún órgano sensorial. El órgano sensorial ordinario parece ser pequeñas áreas con grupos de células nerviosas bipolares esparcidas en el cuerpo y tentáculos. Cuando se someten ejemplares vivos a la acción de la cloretona, los tentáculos son los primeros afectados por la anestesia, siendo los últimos en recuperar la sensibilidad. Los movimientos del cuerpo en conjunto y hasta cierto punto los movimientos de los tentáculos parecen estar regulados por el sistema nervioso.

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THE NERVOUS SYSTEM OF PHORONIDA

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SIX FIGURES

It has long been known that the nervous system of Phoronida was of the surface or epithelial type, such as we find in Echinodermata, Enteropneusta, and some others. Caldwell ('83) was one of the first to describe this condition in Phoronis. He mentions nerve processes in connection with the ectoderm. According to him, both fibers and ganglion cells occur in the surface. Concentrations of nervous tissue occur about the mouth, forming a post-oral nerve ring. This ring forms a semicircular line along the base of the tentacles. Two ciliated pits occur each side of the middle line, and these are considered to be sense organs. On the left the nervous system seems to be continued as a single thick strand near the surface of the body just outside the basement membrane. This rod or strand of tissue Caldwell considers to be a hollow cord. McIntosh ('88) describes a similar condition in *P. buskii*; the general form is the same; the ciliated pits are also recognized; the epithelial nervous system has sense cells and ganglion cells and on the left side is the longitudinal nerve cord or tube, shown but not discussed.

Andrews ('90), in a new species of Phoronis, describes the 'glandular pit' of the lophophore and a "large nerve cord on the left side." He considers it to be solid and surrounded by epidermal cells. According to him, it seems to have a finely fibrillated or possibly a 'coagulated' structure. This nerve cord, so-called, connects directly with the left side of the brain or central nervous system and runs the length of the body. On the right a similar trunk continues but a short distance.

Benham ('89) finds the nervous system immediately below the epidermis as Caldwell was first to observe. Nerve cells and

fibers are recognized in the deeper layers. Some of the fibers are from the epithelial cells at the surface. The nervous tissue follows the epithelium up into the lophophore and into the tentacles. Two longitudinal nerve tubes or nerve bands are described running the length of the body. He suggests that these bands may give the appearance of tubes, due to shrinkage. Epithelial tissue seems to make up these strands, and he did not think they looked like nervous tissue. The suggestion is made that these so-called nerves may be sense organs.

Seyllys-Longchamps ('07) describes the nerve band and the lateral nerve of Caldwell on the left side.

Torrey ('11), in *Phoronis pacifica*, gives a similar account of the nervous system with the following exception:

"The two longitudinal cords which are of exceedingly unequal length, instead of crossing in the nerve ring of the lophophore, are continuous across the middle line at the level of the median mass of ganglion cells. The loop thus formed is closely applied to the latter and touches the lophophore nerve on each side of the rectum, apparently without fusing at either point."

Pixell ('12) in *P. vancouverensis*, describes the usual band of nerve tissue at the base of the lophophore and the nerves up into the tentacles. He mentions the large ganglionic mass on the dorsal surface with the nervous tissue at all places in intimate relation to the inner ends of epithelial cells. In some sections he finds two small lateral nerve cords along the right and left sides of the body. These he says are short and composed of 'punctated' substance.

Nervous tissue was found in the center of the pit at the proximal end of the body and also along the alimentary canal on the outer side of the epithelium, especially in the region of the oesophagus opposite the nerve ring. This area has been suggested as an organ of taste.

In *Phoronopsis hameri* a similar condition of the nerve ring was described, but the ring is narrower and more elongated. There is a conspicuous cord down the left side. Its center is clear and on its border nerve cells are described.

Harmer ('17), in *Phoronis ovatus*, gives the position and form of the nerve ring which is thickened on the dorsal side.

I have had opportunity of examining a considerable number of two species of this interesting group. One, *Phoronis pacifica*, occurs at various places off the coast of southern California, where it has been obtained and examined during three summers. The other, a species of *Phoronapsis*, has been obtained during two years at Monterey Bay and one year at Moro Bay. Both species were studied in serial section. In spite of the occasional difficulty with sections due to the presence of sand grains, a number of perfect series was obtained. *Phoronapsis* also was observed in the field at Monterey and through the kindness of Doctor Fisher at the Hopkins Marine Station at Pacific Grove. These forms were also encountered in even greater numbers at Moro Bay.

The animals do not have many activities. In quiet waters the tentacle whorls are fully expanded just above the surface of the sand. Shadows or bright light seem not to affect them in such positions. Moderate movements in the water made by a pipette near caused them to change their positions. Sand grains dropped gradually upon the expanded tentacles produce little or no contraction. Touching the tentacles seemed not to bring about any marked movements, but if the surface was lightly touched just below the whorl of tentacles there was an immediate and violent reaction and the animal may withdraw within its sandy tube. Any jarring of the sand supporting the sand tubes may also cause this contraction of tentacles and the withdrawal of the upper portion.

In the laboratory the movements of *Phoronapsis* were found to be as follows:

1. A contraction of the tentacles and a shortening of the animal. This is noted whether the animal is inclosed in its sand-grain tube or entirely outside.

2. When out of the tube the whole stem may be waved about. Within the tube the bending is limited to a small area near the tentacles, but outside the tube all parts of the body are capable of bending.

3. Slow contractions when the animal is stimulated outside of its tube.

4. Quick contractions and slower expansion of the tentacles by separation and by matting together.

In the laboratory the reactions to light were not apparent, nor was there any response noted to flashing light. When touched or jarred the response was noticeable, and in the laboratory when the animals were removed from the tubes there was a response to currents. When specimens were changed in fresh sea-water the tentacles tended to separate. This may have been almost purely a mechanical reaction.

When the tubes are suspended upside down there seems to be a little reaction to gravity; the stems tend to turn the oral end up. By changing the position, the animals partly extended from the tubes may be caused to rotate the stems through 360° .

In general, the tentacles have little power of movement. The whole whorl may expand or contract, but there is little evident movement of the individual tentacles. When placed in solutions of chloretone the tentacles tend to separate at once. This takes place a number of times in succession as the animals are changed back and forth, but in the end they remain expanded and die in that condition, but even when apparently dead in some cases they may at once expand when again placed in the chloretone solution. In solutions which are not fatal, the stem or body seems to be the last to be stopped and the first to recover. Much of the extension of the tentacles is purely a sort of floating out; a change of water does this a little, but chloretone and even weak acetic acid may bring about the same results.

In at least one experiment after chloretone, the power to really expand the tentacles was recovered. A real response of the tentacles after a very weak chloretone solution is rather rare, but I believe it does occur in addition to the purely mechanical floating out of the tentacles. When once the animal is killed by weak acid or in some other manner the tentacles no longer float out in chloretone or in any fluid.

Methylene blue was used in various strengths with the living animals and some fairly good results obtained. When not stained too long, bipolar sense cells of a typical sort were seen in the tentacles and at their bases.

The central nervous system was demonstrated as a more or less semicircular band which was not very wide. Some scattered cells in the central nervous system and at the bases of the tentacles seemed to be true nerve cells. All over the surface of the body little groups of stained cells are the sense cells. These are similar to those which Retzius describes in some invertebrates. In the body the sense cells were not as well seen as in the tentacles because of the thick opaque regions where they occurred. The stain was especially valuable in distinguishing the sensory cells in the tentacles and in little groups over the surface of the body. Some nerve strands and cells together with a portion of the central nervous system were merely indicated. More intense stain added nothing to the knowledge of the nervous system; on the contrary, the coloration of connective-tissue cells and others obscured the results obtained with nerve tissues.

The central nervous system of *Phoronis pacifica* is much like that described in other forms. It has its chief concentration a little below the level of the anal opening and the nephridia. This thick part of the nervous system is directly continuous with the epithelium of the surface of the body and dorsal to the anal papilla, and about the depression caused by it. From here the thickening passes towards the tentacles with fibers to them and to the lophophore. Between the depressions on each side are the chief thickenings of the nervous system. Although this central part is continuous with the epithelium, there are distinct nerve cells and fibers. At this greatest thickening there are three chief cell centers among the fibers. There is quite a thickness of nervous tissue about each depression of the lophophore. On the left side near the lophophore is the beginning of the clear cord of unknown function first noted by Caldwell. It is partly surrounded by cells and runs ventrally until it passes through the basement membrane of the body-wall and comes to be just under the epithelium. It runs the length of the body in this position, becoming smaller and smaller. It does not seem to be of nervous tissue, although it is directly connected with the nervous system.

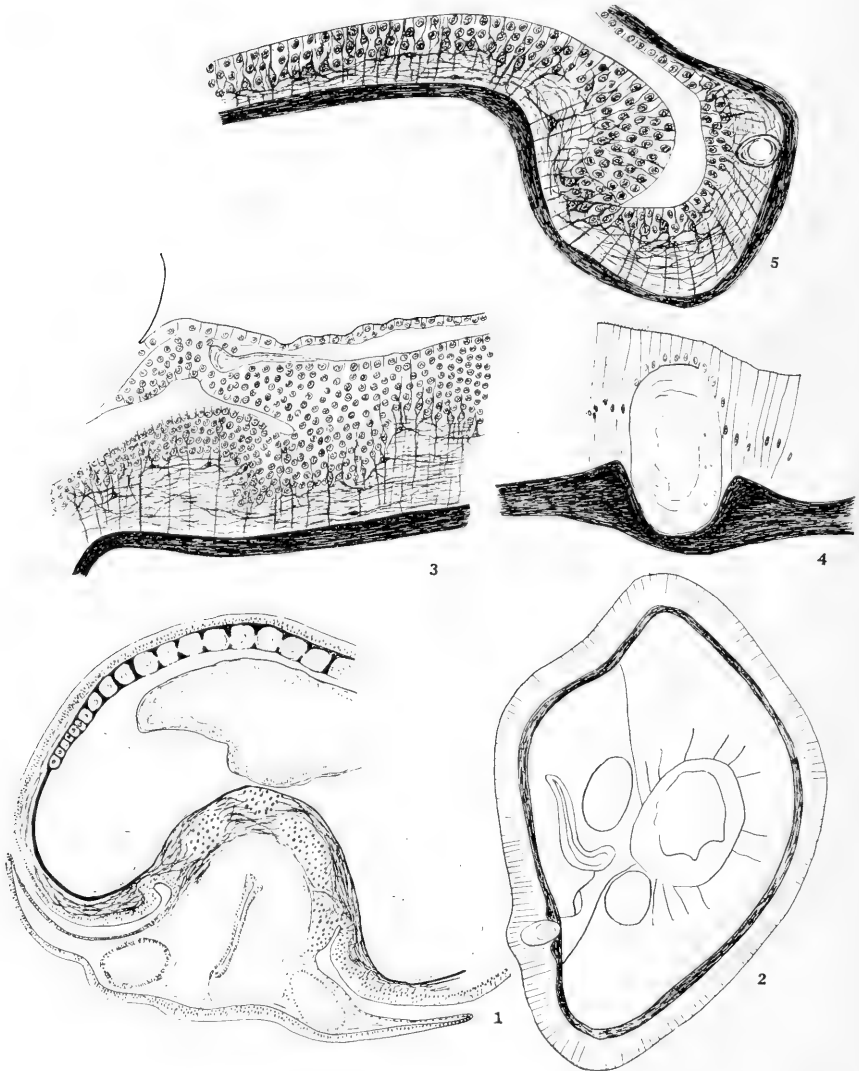
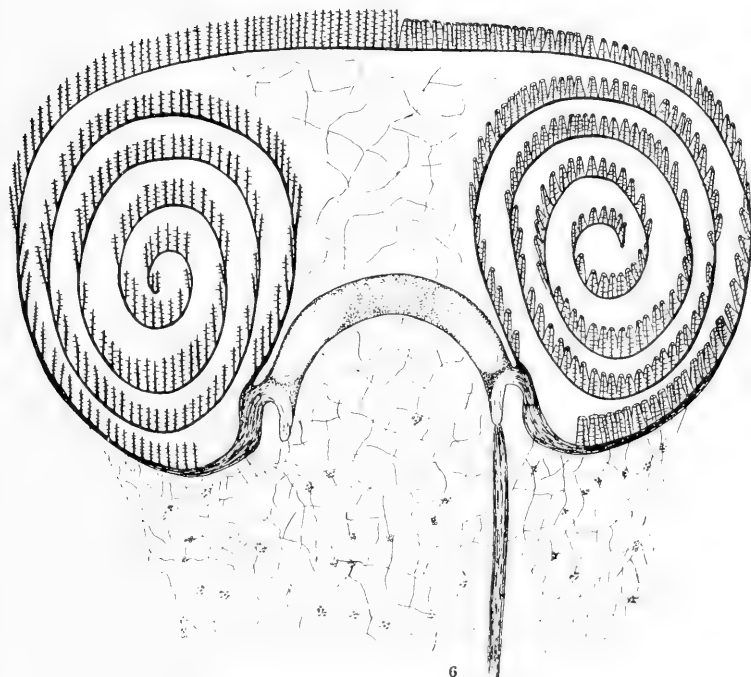


Fig. 1 Section through the body of *Phoronis* below the tentacles and at the level of the central ganglion. The drawing is not completed on the right, but the whole extent of the nervous system is shown. $\times 70$.

Fig. 2 Section across the body of *Phoronis* some distance below the level of figure 1. The long sense organ or left nerve trunk is shown on the left below. In this figure, as in the last, the basement membrane is indicated by a dark band. $\times 70$.



6

Fig. 3 Section across left side of the junction of the brain and the lophophore ganglion. The brain portion is on the right side of the drawing; the lophophore thickening is at the left. The beginning of the longitudinal sense organ is near the upper part of the sketch. The basement membrane is shown as a dark band. $\times 300$.

Fig. 4 Section of the longitudinal nerve trunk. $\times 300$.

Fig. 5 Another view of a section of the lophophore thickening. $\times 300$.

Fig. 6 Diagram to illustrate the general form of the nervous system in Phoronis. The central nervous system is indicated in the central part of the figure. The left nerve strand or sense organ is shown on the right of the drawing. The tentacles are shown cut off on the right side. On the left side the nerves to the tentacle bearing ridge and to the tentacles are shown. The mouth and anal openings are not shown. The central nervous system is a mere band of tissue, but the view is such that its greatest breadth and thickness are shown. $\times 70$.

Torrey ('01) gives a description of the nervous system of this form from sections, but his material must have been poorly fixed, for in well-preserved specimens the central nervous system is continuous laterally with the lophophore organs as well as with the lateral longitudinal cord.

The best results from the study of serial sections were obtained after Flemming or mercuric chloride fixation followed by one of the haematoxylin methods. In well-preserved specimens the general structure of the neuro-epithelium was well shown. Besides the usual epithelial cell, there were three other types recognized: the bipolar sense cells, especially shown in methylene blue in various parts of the body; the supportive cells with the slender fibers running through the thickness of the epithelium and nerve fibers layer, and the nerve cells partly in the epithelium, partly in the fibrous portion. The fibers run laterally at various levels.

CONCLUSIONS

1. The nervous system of *Phoronis* is only partly separated from the epithelium.

2. There is a distinct center or a central nervous system where fibers and cells are more abundant.

3. The lophophoral organs are well developed and may be sense organs.

4. The left longitudinal cord, which may not be nervous tissue, is distinctly connected with the central nervous system. If not a nerve structure, it must be some sort of sense organ.

5. Tentacles and body have bipolar sense cells in little groups.

6. The nervous system controls the muscular system as shown by the use of anesthetics. The tentacles recover last and are affected first by chloretone.

7. The movements are: *a*) Ciliary currents on the tentacles probably not under nerve control. *b*) Contractions of the tentacles at least partly under nerve control. *c*) Contractions of the body stimulated through the surface of the body at almost any point, especially by tactile stimuli just below the tentacles.

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Abstracted by Nils Holmgren, author
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Points of view concerning forebrain morphology in
lower vertebrates.

This paper contains an attempt to show that the pallium of practically all vertebrates is divided into three parts, known from reptiles and mammals as hippocampal, general pallial, and pyriform cortex. This opinion is founded especially on the conditions in embryonic shark brains. This same subdivision is also demonstrated in ganoids and bony fishes, where the so-called striatum is shown to contain these same parts. The everted forebrain of bony fishes is explained as resulting from a preceding phylogenetic inverted and evaginated stage. Also to the sub-pallial parts author has paid attention and attempted to establish the homologies through the series of lower vertebrates. The paper ends with a part treating with the forebrain phylogeny in the light of paleontology. The paper deals in more detail with the forebrains of Petromyzon, Acanthias, Chimera, Protopterus, Polypterus, Lepidosteus, Amia, Acipenser, and Teleosts.

POINTS OF VIEW CONCERNING FOREBRAIN MORPHOLOGY IN LOWER VERTEBRATES

NILS HOLMGREN

University of Stockholm

FORTY-TWO FIGURES (NINE PLATES)

For some years I have been occupied with investigations upon the brain structure of different vertebrates. I have paid much attention to the morphology of the forebrain, which seems to me not sufficiently understood by former workers. I have thus made extensive studies on this part of the brain in Myxine and bony fishes. In addition, I have made studies on Petromyzon, selachians, holocephalians, ganoids, amphibians, reptiles, and mammals, studies which, however, have not yet resulted in publications, but have nevertheless given many valuable points of view concerning forebrain morphology, some of which are subjects of special investigations at this institute and under my direction.

In vertebrates there are two different types of forebrain present, the everted and the inverted. The everted is confined to Polyp-terus, ganoids, and teleosts, the inverted to all other vertebrates. The essential difference between these two types is, that in the former the dorsal walls of the forebrain are bent outward, while in the latter they are bent inward so as to form the roof of the brain. In the everted forebrain the roof is made up by the greatly expanded tela.

Concerning the inverted type, it may be accentuated that the process of inversion, that takes place in a very early embryonic stage and is very soon followed by an evagination process, results in the formation of the hemispheres. This evagination process not only widens the brain cavity in the lateral direction, but also evaginates the lateral ventricles forward and backward to form

the anterior and the posterior horns (if present) of the lateral ventricles. The anterior ventricle extends into the olfactory bulb and forms the ventricle of the bulb. Through the inversion the dorsal parts of the embryonic lateral plates of the forebrain rudiment ('Anlage') not only are brought in contact with each other, but also are sometimes bent down parallel with each other to form in the roof an incomplete septum, dorsal to the foramen monroi, between the lateral ventricles. Through the anterior evagination, parts of the brain vesicle, which hitherto were situated on the lateral side, are brought into medial position in front of the lamina terminalis to form a forward continuation of the imperfect dorsal septum mentioned above. The anterior septum will thus be complete. As there is in the forebrain vesicle a roof- and a floor-part, the perfect septum must be composed of corresponding parts: the roof-part is the medial part of the archipallium (if differentiated), the floor-part is the paraterminal body or septum pellucidum of the authors. The limit between the floor-part and the roof-part is in many cases a very distinct one, a *zona limitans* (in teleosts, selachians, ganoids, amphibians, reptiles, birds, and perhaps also in mammals). It seems to be very unnecessary to make these statements made several times before, but in a recent paper Lundahl has declared that the septum pellucidum (paraterminal body) is a pallial structure, that he names *palaeopallium*. This curious view, which is not consistent with well-known facts, seems to fall to the ground in view of the above statements concerning the evagination of the hemispheres.

As to the everted brain type, it may be accentuated that this is surely not primary in character, but rather to be derived from an inverted and evaginated type. This seems to be evident *a priori* from the fact that the brain of the lower vertebrates (cyclosomes, selachians) is inverted; and also in the organization of the everted brain there are present the most distinct signs of its origin from an inverted type. I will call attention at once to the fact that in ganoids and certain bony fishes (*Lophius*, *Anguilla*) there is a ventricle in the olfactory bulb. This ventricle is continued backwards by a groove which caudad decreases

in distinctness. This groove undoubtedly represents the remains of a previous lateral ventricle and represents the sulcus limitans externus, whose most characteristic feature is its leading out into the bulbar ventricle.

In *Acipenser* and other cartilaginous ganoids the eversion of the telencephalon is very much less pronounced than in bony ganoids and teleosts, and the bulbar ventricle is much greater. This fact seems to point in the direction that the inversion is a primary character of the forebrain of fishes. The same conclusion must be drawn from the fact that the forebrain vesicle of a young embryo of *Lepidosteus* is very clearly inverted.

After these introductory notes, we will turn attention to the pallial structures. What is, then, the pallium in the morphological sense? It is not easy to define this idea in a manner to include therein the pallium of all vertebrates. It seems not to be possible to accept the definition of Edinger and others, whose opinion is that a true pallium (*Episphaerium*=*Neencephalon*) does not receive olfactory fibers of lower order than the third. I cannot accept this view, since it supposes that a brain nucleus would not be able to change its connections without changing its morphological value. It is a well-known matter of fact that the connections of a nerve-nucleus can change in different closely related species (for instance, the nucleus rotundus in bony fishes). But none will for that reason declare that this nucleus is not a homologous one. While the hind part of the roof of telencephalon in selachians is said not to receive olfactory fibers of the second order,¹ but only such of the third, the conclusion is not at all justified that this hind part has a quite different morphological value than the anterior part of similar construction, but with secondary olfactory tracts ending therein. In such a case the conclusion indicated by the facts is that a process of differentiation has taken place in such a manner that the brain roof (the pallium) has become divided into two parts, of which the one is connected with secondary olfactory fibers, the other with tertiary. The morphological unity of the roof remains nevertheless as before.

¹ My studies have shown that this is a false statement, as there are really olfactory fibers of the second order ending in the posterior part of the pallium.

A subdivision of the telencephalon into a hyposphaerium and an episphaerium, of which the hyposphaerium receives secondary olfactory fibers, the episphaerium tertiary, seems not to be a good morphological subdivision. How, then, shall we make a morphological subdivision of the forebrain, how delimit the pallial structures from the subpallial? I think that the morphological ground for such a separation is given in the presence of the fovea limbica and the sulcus limitans externus, or better the sulcus limitans pallii (if present) on the lateral side of the brain-wall and the sulcus limitans medialis and the sulcus limitans (Elliot Smith) on the medial side and, of course, in the presence of the zonae limitantes, those sharply defined, cell-free zones which cut through the brain-wall in the neighborhood of the above-mentioned sulci. *All parts dorsal to the zonae limitantes belong to the pallium, the parts ventral thereto are subpallial.* This definition coincides essentially with that given by Herrick,² but he has not used the zona limitans as limit, but taken an imaginary line connecting the fovea limbica with the sulcus limitans externus and the sulcus limitans medialis with the sulcus limitans (Elliot-Smith). Sometimes this imaginary line coincides with the zona limitans, sometimes it falls a little ventral to the zona. This difference seems to be of very little practical importance and in cases where the zona is lacking, the imaginary line will serve as well as a zona.

CYCLOSTOMATA

Petromyzonts

The forebrain of *Petromyzon* is very clearly inverted, but the medial borders of the inverted parts do not reach each other. Thus a pallial septal structure does not occur. Lateral ventricles are evaginated cephalad in the olfactory bulb, caudad in the 'olfactory lobe.' In adult *Petromyzon* ventricular grooves are absent, but in the larva there is a distinct sulcus limitans externus (fig. 1, *s.l.e.*) leading cephalad into the bulbar ventricle.

² For literature see the list in my paper: *Zur Anatomie und Histologie des Vorder- und Zwischen-hirns der Knochenfische. Hauptsächlich nach Untersuchungen an Osmerus eperlanus.* Acta Zoologica, vol. 1, 1920, pp. 137-315.

Through this sulcus the pallial parts of the brain are approximately separated from the subpallial. The pallium is medially bordered by the primordium hippocampi of Johnston (praethalamus of other authors, fig. 2, *p.h.*). I wish not to express any definite opinion as to this primordium hippocampi. Johnston's opinion is founded on the behavior of the velum transversum, but there seems to be some analogy between the primordium hippocampi and the eminentia thalami of amphibians, especially in the relations to the taenia bundles. Also the embryological development of this hippocampal formation is not convincing to the advantage of Johnston's interpretation. For the 'primordium hippocampi' of the *Ammocoetes*-larva of 30 mm. is an ependymal border of the medial pallium—a border that insensibly passes over into the ependymal roof of the brain-vesicle (fig. 2, *p.h.*). Later the primordium grows thicker (fig. 3A) as the taenia bundles enter it, and it becomes ganglionic. The fact that the 'primordium hippocampi' is situated before the point of lateral attachment of the velum transversum is not a fact of definite importance, since there is no ground present why the di-telencephalic border should be a straight line behind the 'primordium hippocampi' and not a bent line before this part of the brain. A comparison between figures 3A and 3B, respectively from *Ammocoetes* and *Triton*, reveals so great concordance that I find the opinion of the 'primordium hippocampi' as a part of thalamus comparatively well settled.

I thus regard as the pallium of *Petromyzon* what Edinger calls the lobus olfactorius. In the cephalic part of this lobus olfactorius he observed a small dorsomedial part, which he thought to be an 'Episphaerium-Anlage' (fig. 4, "*Ep.*"). This part is a great olfactory glomerulus with dorsocaudal situation in the olfactory bulb. (In the figure in his 'Vorlesungen,' which illustrates the 'Episphaerium' rudiment, the 'primordium hippocampi' of Johnston is falsely labeled 'ganglion habenulae'.)

In the pallium there is present a cortical layer of pyramidal cells (fig. 2, *Cort. 1*). This layer is somewhat diffusely composed, but nevertheless very conspicuous. It is laterally somewhat subdivided, but the subdivisions have rather the appearance of

detached cell-groups than of special cortical areas. Thus one can state for *Petromyzon* that there is a cortical layer present extending from the ventro-lateral part of the pallium to the medial. Such a cortex I would be very inclined to call a palaeocortex, but this term is preoccupied by Kappers in another sense.

At the ventricular wall there is a second layer of cells, forming a ventricular cortical sheet (fig. 2, *Cort. 2*).

The entire hemisphere receives secondary olfactory fibers from the olfactory bulb, but the pallial part receives also tertiary olfactory tracts from the subpallial part of the forebrain. There is no part present exclusively arranged for the reception of the tertiary tracts only. Under the exterior cortical layer there is a stratum containing unmyelinated fibers.

The limit between the pallial part and the subpallial is not marked by a zona limitans, but nevertheless the limit is very conspicuous, the cells on the opposite sides of the limit being of different dimensions, the dorsal larger, the ventral smaller, but more densely crowded.

In the subpallial part it is impossible to distinguish different subdivisions in the arrangement of the cells. The entire basal part of the brain acts as a tuberculum olfactorium + striatum + nucleus taeniae, but no one of these parts is differentiated.

Is the forebrain of Petromyzon of ancestral type? Almost every account of forebrain phylogeny begins with the petromyzonts as having the most primitive structure of all living vertebrates. This conception is to some degree justified by the, in many respects, ancestral character of these animals and also by some features in the forebrain morphology, the forebrain being more simply built up than in other vertebrates. There are no special subpallial nuclei and the pallium is uniformly constructed. But are these features sufficiently significant to allow the conclusion that the entire forebrain is of an ancestral type? I think not, and I will now try to show that such a presumption is not very well founded. I will try to show that the forebrain of *Petromyzon* is secondarily reduced.

The ventricular system of *Petromyzon* consists of a medial unpaired ventricle from which the lateral ventricles rise. Each

lateral ventricle splits up into two parts, a rostral and a caudal one. The first is the ventricle of the olfactory bulb (fig. 4, *o.v.*) homologous with the same in other animals. The second (figs. 1, 2) has been compared with the lateral ventricle of the hemisphere and regarded as homologous with this. The common aperture of the 'lateral ventricles' into the medial ventricle has been regarded as the foramen monroi. This conception of the *Petromyzon* forebrain is not allowable, as will be clearly demonstrated by means of an examination of the ventricular system in an *Ammocoetes* of 1.7-cm. body length. In this stage the forebrain has the same exterior aspect as in more advanced stages and in adult animals, but the ventricles are arranged in another way. The bulbar and the hemispherical ventricle are free from each other (compare figs. 4, 2, and 1), opening into the medial ventricle with separate openings, the bulbar lying ventral to the hemispherical. From the bulbar ventricle a distinct groove runs backwards in the wall of the medial ventricle (fig. 1, *s.l.e.*) and a good distance under the opening of the hemispherical ventricle. This groove apparently is the sulcus limitans externus, as pointed out above. This furrow in other vertebrate brains is seen at the lateral wall of the lateral ventricle in various positions in different vertebrates, but always belonging to the lateral wall of the lateral ventricle. According to the current view of the ventricular system in *Petromyzon*, the sulcus limitans externus here should not belong to the lateral ventricle. It is highly improbable that *Petromyzon* should in this respect represent a quite different type than all other vertebrates. Thus I am obliged to accept the opinion that the hemispherical ventricle in *Petromyzon* represents only the dorsolateral part of an ordinary forebrain ventricle. This dorsolateral part is tube-like, directed towards the neck part of the pallium, perhaps representing a ventricle analogous to the posterior diverticulum of the forebrain ventricle in *Acanthias*. Owing to this, it is clear that the foramen monroi of the *Petromyzon* brain is no true foramen monroi. The ventricular system in the forebrain of *Petromyzon* thus is to be considered as a highly reduced one. This reduced ventricular system indicates that also the entire forebrain is not ancestral, but

secondarily modified. How the primary cyclostome forebrain originally was built up, it is not possible to make out in detail, but that the ventricular system of it was wider, with normal lateral ventricles and a sulcus limitans externus as in other brains, is sure. The general form of such a forebrain may have been more or less like that of selachians with the bulbi olfactori situated lateral at the rostral part of the forebrain vesicle, as in an early embryo. The hemispheres were surely not joined in the dorsal mid-line, where the brain roof consisted of a tela, more or less as in *Chimaera*. It is a priori probable that the transformation of the exterior of the forebrain was accompanied by transformations in the finer structure, but the nature of these transformations it is not possible to determine. In a young *Ammocoetes*, however, it is clearly seen that there are two pallial cell-layers present, a peripheral and a ventricular. These layers are in the medial part distinctly separated by a relatively cell-free space. These two layers are also to be distinguished in adult animals, but the cell-free space here is absent. Perhaps the conclusion from this fact may be admitted that there were two cortex layers present in the ancestral cyclostome, as in selachians, *Dipnoi*, etc., as will be shown later on.

Myxinoïds

The conditions in the forebrain of *Petromyzon* are repeated in *Myxine*, as I have already pointed out in my memoir on the remarkable brain of this animal (*Svenska Vetenskapsakad:is Handlingar*, vol. 60, no. 7, 1919). Through the reduction of the ventricle system and the hyperinversion of the pallial parts, the curious aspect characterizing this brain is realized. Here I may only call attention to the fact that the pallium contains a very distinct cortical layer. No zona limitans is present and it is difficult to limit the pallium from the subpallial parts, the cells of both being equal in size and the basal cortical cells flowing over into the subpallial. In the subpallial parts no separate ganglia are to be recognized, as in *Petromyzon*.

For particulars reference may be made to the above-quoted paper; here it is enough to accentuate that the principles of the brain construction are the same in *Myxine* and *Petromyzon*.

SELACHIANS

In selachians the inversion of the forebrain is much more advanced than in *Petromyzon*, the inverted parts reaching each other in the medial plane and there being bent down and grown together to form the short dorsal septum hanging down between the lateral ventricles in the region of the foramen monroi. The evagination also has a different character than in *Petromyzon*.

The lateral ventricle is evaginated into the olfactory bulb, which has a lateral situation, but to this there is added an evagination in the rostral direction which causes a ventricular pouch medial to the olfactory crus. In consequence of the latter evagination, brain parts, occupying in other vertebrates a medial position in the septum, should be brought forward and lateralward to cover the topographically foremost part of the brain. Morphologically, the frontal parts of the brain hemispheres thus should belong to the medial side—a matter of fact that should be of some importance in comparing the selachians with other vertebrates. The reason why I have here not followed this scheme is this:

This scheme is only applicable under the presumption that the terminal situation of the olfactory crus is primary. But early ontogenetic stages not only in selachians, but also in cyclostomes, ganoids, teleosts, dipnoans, and amphibians, show that the primary situation of the bulbus is a lateral one and that in these animals except in selachians, the bulbus rudiment is dislocated during ontogeny to a terminal position. As the bulbus rudiment is a subpallial structure, this wandering of the bulbus rudiment is generally effected in the subpallial part without changing the pallial conditions. In early ontogenetic stages the rudiment of the pallial cortex is found medial and in its foremost part rostral to the bulbus rudiment, in the same way as in selachians, as shown below. Thus I consider the selachian condition to be primary; those of the other vertebrates, with the hippocampal formation extending directly to the olfactory crus, as secondary.

The pallial structures in selachians seem to have been very little understood and as to the cortical layers they have been nearly

unknown. I, therefore, here will treat of them at some length. In the adult *Acanthias* these cortical structures are not very conspicuous, owing to the fact that the cells of the different cortical parts are not very different in aspect; but in embryos of different ages it is very easy to demonstrate that real cortical layers are differentiated. But these structures are already in the postembryo not very conspicuous, owing to the increase of ganglion cells from the ventricular sheet of neuroblastic tissue, and in the adult animals they are scarcely more than indicated by more or less packed cell formations. To make these structures understood it is necessary to follow their embryological development by means of series of different stages.

My material consists of the following stages of the common *Squalus acanthias*: 3, 3.3, 3.7, 3.8, 3.9, 4, 4.5, 4.9, 5, 6.5, 8, 15 cm. length of the body. The brain was dissected out and cut (15 to 20 μ), especially in the transverse direction. Staining with cresyl-violet.

1. *Stage of 3-cm. body length.* In this stage in the pallial part of the brain the ventricular layer of neuroblastic cells is in great activity forming nerve cells which are pressed outward to form a thick sheet covering the whole dorsal aspect of the ventricle. This layer is not yet delimited from the neuroblastic layer from which it is derived. No differentiation is to be found in this primordial cortex.

In the basal parts of the forebrain the cortex of the tuberculum olfactorium (area superficialis basalis of Johnston) is about to delaminate from the ventricular position in order to form a separate area of cortex-like structure.

2. *Stage of 3.3-cm. body length (measured after fixation in Carnoy's fluid).* The primordial cortex is in the stage of delamination from the ventricular layer. In the foremost part of the brain the primordial cortex is continuous with the thick ventricular layer, except in its lateral part, where there is a thin cell-free space between it and this layer (fig. 5). The delamination of the dorsal and medial part of the cortex is not so far advanced, but a relatively cell-free space between the cortex and ventricular layer is marked by a sheet of small cell-free spots which are

seen in the sections as a row of white points (fig. 6). Thus the primordial cortex here is practically differentiated as an uninterrupted cortical layer passing from one side of the brain roof over to the opposite. Examining this layer with attention, one cannot avoid observing that the extreme lateral part of it is somewhat different from the remaining. Its cells are a little larger, less densely packed and assume a paler color than the other cortical cells. *In this early stage the differentiation of the pyriform cortex thus is begun.* This pyriform lobe is quite dorsal in position and dorsal also to the olfactory prominence (bulbus olfactorius). Lateral to the pyriform lobe a relatively cell-free space represents an obsolete zona limitans, lateral to which the olfactory bulb rudiment is situated.

It is of some importance to observe that the primordial cortex at one point is continuous with the ventricular layer, viz., the dorsolateral part, medial to and below the pyriform lobe, where cell masses are streaming out from the ventricular layer pressing the primordial cortex against the brain surface and partially coalescing with it. This process represents the first stage of the formation of a new cortical layer, which begins below the medial part of the pyriform cortex and from here extends medially, forming what I will name the general pallium.

In the subpallial parts of the brain the cortex of the tuberculum olfactorium is nearly quite disengaged from the ventricular sheet. The bulbar nuclei are in this stage under formation from the ventricular layer at the lateral part of the ventricle inside the olfactory bulb rudiment (the most lateral part of the forebrain). Between these bulbar nuclei and the upper border of the tuberculum olfactorium cortex there is found a diffuse, in the lateral part rather dense, mass of cells, derived from the underlying ventricular sheet. This cell-mass, I think, represents a lateral olfactory nucleus.

3. *Stage of 3.7-cm. body length (measured after fixation in Carnoy's fluid).* In this stage the pyriform lobe is separated from the remaining primordial cortex layer, excepting in the foremost part of the brain, where the two cortex components seem to flow together. The part of the ventricular layer from which the

general pallium is under formation is much broader than in the preceding stage and occupies nearly the whole dorsal aspect of the ventricular layer (fig. 7, *g.p.c.*). From this cell-masses are streaming out below the primordial cortex (fig. 8, *prim.c.*), pressing the intermediate part of this cortex (the exterior layer of the general pallium) against the brain surface and partly confluent with it (fig. 8, *g.p.c.*). Since in the foremost part of the brain the lateral and medial parts of the primordial cortex are not exposed to this pressure, the primordial pallium is thus divided into three parts, a medial, a dorsal, and a lateral, respectively corresponding to the hippocampal, the general pallial, and the pyriform cortex, of which the pyriform is formed first. The general pallium (the inner layer) develops after the pyriform lobe and causes a frontal separation of the hippocampal pallium from the pyriform lobe.

The hippocampal part of the primordial cortex rostrally is tolerably well differentiated. At the recessus neuroporicus the hippocampal pallium dorsal to this recess *joins the corresponding part of the other hemisphere*. Caudal to the neuroporic recess the hippocampal part is continuous with that of the other side, forming a thin sheet of cells passing the mid-line close to the ventricular layer. On each side of the mid-line the hippocampal cortex is confluent with the underlying general pallial rudiment. In summary, the hippocampal primordial pallium consists of: 1) a frontal part covering the medial part of the frontal pole of the brain hemisphere; 2) a commissural part dorsal to the recessus neuroporicus.

In the front part of the brain the zona limitans lateralis is well marked. At the extreme lateral part of the forebrain the zona limitans is obsolete and the pyriform lobe is continuous with the lateral olfactory nucleus. Caudal to the bulbus olfactorius rudiment the zona grows more conspicuous.

The evagination of the forebrain in this stage not being more than commenced, the septal structure is very short. The zona limitans medialis is not pronounced and the subpallial parts directly adjoin the hippocampal structure at the upper level of the neuroporic recess. The septum is filled with a dense mass of

cells in which no differentiated nuclei are to be seen. This cell-mass is rostrally continuous with the lateral olfactory nucleus.

The cortex of the tuberculum olfactorium is well developed and caudally merges with the preoptic nucleus or the somatic area of Johnston. The nucleus olfactorius lateralis is denser than in the preceding stage.

4. *Stages of 3.8 to 4.5-cm. body length.* In the stages of 3.8, 3.9, 4, and 4.5 cm. the development is not much advanced in relation to the stage of 3.7 cm. body length. The brain has grown somewhat larger and the cells migrated from the ventricular layer are more numerous, but the general arrangement is almost the same as before. In the stage of 4.5 cm., however, it is to be noted that the pyriform cortex is much better limited against the ventricular sheet and in the medial part of the forebrain also against the general pallium rudiment. In the foremost part of the forebrain, the pyriform cortex and the hippocampal cortex rudiment are everywhere continuous with each other (fig. 9). The zona limitans lateralis is very conspicuous in the cephalic part of the brain (fig. 10, *z.l.l.*); it grows, however, more indistinct in the caudal part, behind the olfactory bulb rudiment. A zona limitans medialis is not to be seen.

5. *Stage of 4.9-cm. body length.* Of this stage I have had at my disposal only a series of transverse sections which were inclined forward. Thus the cortical parts of the forebrain are not in the same position as in the perfectly transverse sections described before. Therefore, it is somewhat difficult to make an adequate comparison between this stage and the preceding ones. But so much is at once clear, that no greater changes have taken place. The arrangement of the layers is quite the same. The part of the primordial cortex, however, uniting the pyriform lobe with the hippocampal rudiment has grown more conspicuous, a cell-free space in the foremost part of the brain having arisen between this cortex and the general pallial rudiment which now seems to be in rapid development.

The evagination of the forebrain being much more advanced, the septum has increased considerably in length and the septal nuclei are under formation. In the preceding stages the septum

is filled up by an elongate, vertical cell-mass belonging to the nucleus olfactorius lateralis, which basally merges with the tuberculum olfactorium cortex. The cells of this septal nucleus, however, assume a much paler color than the tuberculum cells. In the stage of 4.9 cm. the septal nucleus consists as before of a dense cell-mass on each side of the medial line and begins basally to detach from the tuberculum cortex. Between the two septal nuclei, the mid-part of the septum is filled up by rather scattered cells. Ventrally this cell-lamina spreads out between the medial parts of the tuberculum olfactorium cortex. Against the foramen monroi where the septal nuclei are confluent below the neuroporic recess the vertical cell-lamina disappears, and the basal part of the same is found ventral to the united basal parts of the septal nuclei.

The inversion of the forebrain vesicle is not essentially more advanced than before. The zona limitans lateralis is tolerably well marked, but the zona limitans medialis is not visible at all.

6. *Stage of 5-cm. body length (measured after fixation in Carnoy's fluid).* This stage is much more advanced than indicated by the body length. This was undoubtedly much greater in the living specimens than in the preserved. I think that the difference may be estimated at 3 to 4 mm. in favor of the living specimen.

In this stage the essentials of the pallial structure are differentiated. Therefore I will deal with it in a little more detail than with the preceding stages. I here will adopt the method of describing a number of transverse sections, cut at different levels of the forebrain.

A. Cross-section taken just in front of the foremost part of the lateral ventricle (fig. 11). The wall of the ventricle is just touched by the section. At this level the hemispheres are quite free from each other. The ventricular neuroblastic layer is touched a little below the middle of each hemisphere. Dorsal to this point is a great dense cell-mass, representing the ventricular cell-layer which covers the dorsal portion of the ventricle frontally. Dorsal to this cell-mass is a great cell-free space lying as a

cap on it, and dorsal to this space is seen a dense regular cortical layer (*prim.c.*). This is thicker in its lateral part than in its medial. The cortical layer represents the primordial cortex, the lateral part of which belongs to the pyriform lobe, the medial to the hippocampal rudiment.

Below the ventricle is a clear space crossing over the ventral part of the brain and delimiting the nucleus olfactorius lateralis and the tuberculum olfactorium cortex which lie below it. This space, poor in cells, medially bends up dorsad to the medial border of the hippocampal rudiment. The dorsal part of this clear space in the future development is destined to become the zona limitans medialis (*z.l.m.*). The lateral part of the space in question also bends dorsad to the lateral border of the pyriform lobe and forms the *zona limitans lateralis* (*z.l.l.*). *Thus at the front pole of the forebrain vesicle the zona limitans medialis and lateralis unite.* The above-named cell-free space is present always from the 3.3-cm. stage, but now for the first time it is possible to see the future fate of it.

B. Cross-section taken through the foremost part of the septum (fig. 12). The lateral ventricle is cut in its rostral part, the ventral as well as the dorsal part of the ventricle being opened by the section. The cross-section of the ventricle is more or less 8-shaped. The cell-free space below the ventricle has disappeared but the medial and the lateral parts of it are seen as *zonae limitantes*, of which the medial (*z.l.m.*) is less conspicuous than the lateral (*z.l.l.*). The tuberculum olfactorium cortex covers the whole ventral surface of the section, and is continuous with the septal nucleus mentioned in preceding stages.

In the pallial part of the forebrain the pyriform lobe (*p.c.*) occupies the lateral part and consists of a lateral rounded cell-mass, that sends a cortical lamina in the medial direction parallel to the dorsal surface of the brain vesicle. This cortical lamina is medially continued by a thin sheet of scattered cells (*sc.c.*) which medially merges with the hippocampal rudiment (*h.c.*). The lamella of scattered cells representing the outer layer of the general pallium is the backward extension of the cortical part connecting in the preceding sections the pyriform lobe with

the hippocampal. In the section before us this connection is nearly broken off. This condition probably is the consequence of the development of the inner general pallial cortex (*g.p.c.*). In the preceding stages this cortex was quite ventricular in position. Now it is separated from the ventricle by a cell-poor space and has assumed the characters of a thick special cortical layer. The preparations give the impression that the growth of this cortex should be the reason for the breaking of the primordial connection between the hippocampal and the pyriform cortex. Below the general pallium the ventricular neuroblastic layer seems to be in activity, forming new nerve cells, but the activity is not very rapid (the ventricular cell-layer having not increased much during the period from 5- to 6.5-cm. body length) except at one point, viz., medially below the hippocampal rudiment. Here there is a very pronounced emigration of cells (*h.em.*) towards this rudiment. The hippocampal rudiment in this section appears as a nearly vertical, short lamella (*h.c.*) of densely packed cells. Ventrally this cell formation is limited by the obsolete zona limitans medialis, dorsally it insensibly merges into the above-mentioned lamina of scattered cells, connecting it with the pyriform lobe. Laterally the hippocampal rudiment is continuous with the emigrating cells (*h.em.*) mentioned above.

C. Cross-section through the tip of the recessus neuroporicus (fig. 13). The olfactory bulb rudiment is touched at its cephalic side. The most striking feature of this section is that the pyriform lobe (*p.c.*) is quite dorsal in position. The portion occupying in the preceding section the lateral part of the brain vesicle has disappeared and the corresponding part in this section is filled up by scattered cells. A closer analysis of this field of scattered cells shows that it represents the zona limitans, which has bent upward in such way that it is here cut longitudinally (*z.l.l.*). The upper border of this field lies lateral to the pyriform lobe, and in the consecutive sections the zona here bends caudally again on the lateral border of this lobe, above the olfactory bulb rudiment.

The general pallial cortex (*g.p.c.*) covers the main part of the dorsal aspect of the section. Outside of it there are some scat-

tered cells, remnants of the outer general pallial layer connecting the pyriform with the hippocampal lobe.

The hippocampal rudiment (*h.c.*) consists of a rather dense cell-mass, including the above-mentioned ventricular mass of emigrating cells, and a lamella of scattered cells lying medial to it, continuous with the scattered cells on the surface of the brain.

D. Cross-section 60μ before the foramen monroi (fig. 14). The section touches the front surface of the olfactory bulb recess and passes through the basal portion of the neuroporic process.

In the pallial parts the following changes from the preceding section are to be mentioned.

1. The pyriform lobe is, of course, distinct, but its medial border is confluent with the lateral part of the general pallial cortex.

2. The general pallial cortex has grown thicker in its medial part than in its lateral, so as to form in the cross-section a club-like figure, with the thickening (*g.p.t.*) directed toward the medial line. The medial part of this cortex is bent down a little.

3. At the point where the pallial thickening just mentioned ends we meet with a new cell-mass, emerged from the medial emigration (*h.em.*) locus of the ventricular wall mentioned above. From this point there are streaming in, in the dorsomedial direction, a dense mass of cells. These cell-groups cover the flanks of the neuroporic recess.

4. The hippocampal formation, lying in the preceding section on each side of the neuroporic recess, now forms a bridge above the recess, connecting the two hemispheres with each other. The emigrating cell-groups (*h.em.*) of the preceding paragraph seem to force this bridge in the dorsal direction and to press it together to a relatively thin cell-lamella in the median line. The lamella, together with the emigrating cell-masses, representing a second emigration of hippocampal cell elements, I consider as the hippocampal formation.

5. The zonae limitantes are not very conspicuous.

6. The changes in the subpallial parts are not great. The septal nucleus shows two condensations of cells, a dorsal just below the neuroporic recess and a ventral a little above the medial

border of the tuberculum olfactorium cortex. Between the two ventral nuclei the space is filled up by scattered cells.

7. The bulbar nuclei are confined to the bulbar rudiment. The nucleus olfactorius lateralis is a rather dense cell-mass filling up the space between the bulbar rudiment and the tuberculum olfactorium cortex.

E. Cross-section through the frontal part of the foramen monroi (fig. 15).



In the pallial parts the most striking feature is that the hippocampal formation forms an unpaired medial cell-mass (*h.c.*) and that the thickened medial part (*g.p.t.*) of the general pallium cortex (*g.p.c.*) extends medially to form a little more caudally a continuous layer above the hippocampal formation which at the same time decreases to form more caudally a very low cellular ventricular eminence. The cells of the thickened general pallial cortex are not so densely crowded as those of the remainder of the same cortex. Laterally this cortex is confluent with the pyriform cortex (*p.c.*). The zona limitans lateralis (*z.l.l.*) is well pronounced.

In the subpallial parts the ventral septal nucleus mentioned sub C, 6 is found in the medial line between the two cortical layers of the tubercula olfactoria as an unpaired thick ventricular layer of relatively scattered cells. The scattered cells in C, 6 lying between the two nuclei, are found ventral to this unpaired nucleus.

F. Cross-section through the hind part of the forebrain, where the latero-occipital ventricles are disappearing (fig. 16).

In this section the dorsal continuity of the general pallia is broken. The thickened part of the cortex (*g.p.t.*) has grown very greatly and becomes caudally larger and larger so as to fill up the whole dorsal part of the connecting lobe of the telencephalon. The pyriform cortex (*p.c.*) medially is continuous with the general pallial cortex (*g.p.c.*). Laterally it is not well marked from the lateral olfactory nucleus (*n.olf.l.*). Thus a zona limitans lateralis is not to be found in this part of the brain.

The subpallial part has not greatly changed its appearance from the foregoing section. The medial parts of the tuberculum

olfactorium cortex have been replaced by the 'area somatica' of Johnston(?), a cortex-like structure which in the form of a  covers the brain medially. The band of scattered cells in foregoing sections between the medial extremities of the tubercula olfactoria now is found below the area somatica. In preceding sections there is a more densely aggregated group of cells (*n.t.*) at the medial end of the olfactory cortices. More backwards these condensations are found more lateral and as the -shaped area somatica makes its appearance in the series this cell-heap lies below the lateral end of the same area. This cell formation that accompanies and emigrates from the inner part of the tuberculum olfactorium in Cajal preparations has been found to give origin to taenia fibers. It is the nucleus taeniae, hitherto not clearly demonstrated in selachians.

7. *Stage of 6.5-cm. body length (measured after preservation in formolalcohol). Cross-sections.* In this stage no essential changes in the structure of the telencephalon are to be noted. The general arrangement is the same as in the preceding stage, but the different parts are more accentuated than before. Thus the hippocampal rudiment has grown much more distinct with more densely packed cells, especially in its frontal part (fig. 17 A). It now has taken on the aspect of an ordinarily constructed cortical layer. The two hippocampal cortices meet each other above the neuroporic recess.

The medial thickening of the general pallial cortex is greater than before (fig. 17 B) and just at the level of the basis of the processus neuroporicus it joins in the medial line the corresponding part of the opposite side. Here the hippocampal cortex is no longer present.

The pyriform cortex is not distinctly delimited from the general pallial cortex. The zona limitans medialis is absent. The lateral zona sometimes is present, sometimes absent.

The nucleus olfactorius lateralis is more distinct (fig. 17, *n.olf.l.*) than in the preceding stage. Other structures are as before, but somewhat more pronounced.

8. *Stage of 8-cm. body length (measured after fixation in 10 per cent formalin). Cross-sections.* The definitive arrangement of

the forebrain is attained in principle. The inversion of the pallial parts has become much greater than in the preceding stage. The general pallial thickening has been bent down to a vertical position in the upper part of the 'septum' partly between the hippocampal cortex and the ventricular wall. In the subpallial parts a corresponding process has taken place, and the medial part of the tuberculum olfactorium cortex is also brought to a vertical position. The forward evagination of the forebrain vesicle also is much more pronounced, the 'septum' thus having increased considerably in length. At the foramen monroi, where the 'septum' is broken off, the pallial part of it is separated from the subpallial.

1. Pallial parts. The hippocampal cortex is very clearly seen in the front part of the 'septum,' where it is confluent with the pyriform cortex (fig. 18). The upper end of the hippocampal cortex is bordered by more densely arranged and dark-staining cells (fig. 19, *sub.*). This border or cell-lamina lies inside of the medial part of the general pallium (fig. 19, *g.p.c.*), which is bent down to a vertical situation (fig. 10) in the upper part of the 'septum.' The mentioned border has the appearance of a 'subiculum' as found in reptiles. It begins as soon as the separation of the hippocampal and pyriform cortex has taken place and in front of the general pallium.

The hippocampal cortices are joined with each other by a cellular bridge dorsal to the recessus neuroporicus (figs. 20, 21). This seems to be a result of the secondary medial fusion of the evaginated parts of the forebrain, which had already taken place dorsal and ventral to the tube-like recessus neuroporicus.

The general pallial cortex has grown much thicker than before, the medial swelling being now voluminous and sharply bent down medially in the 'septum' (fig. 1, *g.p.t.*). Towards the foramen monroi, where the general pallial cortices are confluent dorsally (fig. 22, *g.p.c.*), the medial parts are very voluminous, causing at the foramen monroi a thick ridge hanging down from the roof of the ventricle (fig. 22). The main part of the 'episphaerium' of Edinger is made up by the general pallium (figs. 22,

23). The cells here form several dense groups connected with more scattered cell-formations.

The pyriform cortex is in its whole extent tolerably well separated from the general pallium (figs. 19 to 23) and from the lateral olfactory nucleus or the bulbus olfactorius nucleus. Yet fusions of all parts are common, but in such cases the cell arrangement or cell-size makes it always possible to make out the limits of each nucleus. Thus it is always possible to find the situation of the zona limitans lateralis (figs. 19 to 23).

2. Subpallial parts. In the septum the pallial parts are separated from the subpallial by a rather obsolete zona limitans medialis and a sulcus limitans internus. The zona limitans medialis passes immediately dorsal to the neuropore. All parts below this zona belong to the subpallial septum.

The subpallial septum is divided into two parts by a very conspicuous sulcus, accompanied in its hinder part by an ependymal thickening. This 'sulcus septalis' (figs. 21, 22, s.s.) begins at the ventral angle of the lateral ventricle. From this it turns obliquely upwards and caudal to the front margin of the foramen monroi, where it is found immediately below the sulcus limitans internus. In the 15-cm. postembryo of *Acanthias* this sulcus septalis is very sharply pronounced. It marks the ventral limit of the nucleus lateralis septi (fig. 21, n.l.s.). At the extreme caudal portion this nucleus sends up a small tongue above the foramen monroi (fig. 22, n.l.s.). *This represents a fimbrial structure at least partly homologous with the fimbria in other vertebrates.*

The nucleus medialis septi (figs. 19 to 21, n.m.s.) is found as in the preceding stages: in front as a cell-lamina directed upward and forming a dorsal continuation of the cortex tuberculiolfactorii. In the caudal part this lamina is divided into a dorsal and a ventral part, of which the first forms an unpaired cell-mass around the base of the processus neuroporicus. The ventral portion, however, diverges from the dorsal, and at the level of the front part of the foramen monroi it is found in the middle of the basal septum, where it is seen in cross-sections as a rather well-delimited 'nucleus' (fig. 22, n.m.s.), lying in the diffusely arranged cell-mass which fills up the basal part of the septum.

The nucleus taeniae (figs. 22, 23, *n.t.*) and the tuberculum olfactorium cortex are not changed in this stage.

The nucleus olfactorius lateralis (*n. olf.l.*) has grown larger and is seen in all sections as a great cellular condensation with somewhat cortical aspect dorsal to the lateral border of the tuberculum olfactorium cortex.

In the lateral wall of the forebrain ventricle there are three 'striatal' swellings, two rostral and a caudal. These represent a striatum. In adult animals these swellings are very strongly developed, containing a ventricular nucleus, described by former writers as 'epistriatum' (figs. 20 to 23, *st.s.*)

9. *Stages of 15-cm. to full-grown animals.* In the further development no essential changes occur. The different parts, excepting the tuberculum olfactorium, however, are growing indistinct. The cortical layers in the pallium lose their characters of well-defined cortices, the cells being spread out and the limits thus being indistinct. This process results in adult animals in a pallium, where it is very difficult to recognize the structures so distinct in the embryo. This condition probably is the reason for the current view, according to which the selachians have no real pallial cortex, except the so-called 'episphaerium' of Edinger. Edinger says (Vorlesungen, 2. Teil, p. 249):

Im caudalen Abschnitte dieses Palliums tritt schon sehr früh, in der ersten Anlage, vielleicht schon bei den Selachiern, sicher bei den Amphibien, eine neue Formation auf, eine Formation, welche keine direkten Riechfasern mehr aufnimmt, wie das Hyposphaerium. Hier münden vielmehr tertiäre Bahnen aus dem Lobus olfactorius. Diesen neuen Abschnitt nennt man Episphaerium. Mit ihm sellt sich zu dem Palaeencephalon das Neencephalon.

Dieses Episphaerium vergrößert sich bei den Reptilien schon so sehr, dass es die Riechnervenendigungen frontaler und lateraler schiebt und zunächst die ganze Medianwand in hinteren Abschnitte und einen Teil der Dorsalwand erfüllt. Immer weiter wachsend erzeugt es dann bei den Reptilien, den Vögeln und namentlich bei den Säugern im wesentlichen das, was als Vorderhirnmasse zunächst auffällt. . . . Minimal in seinen Anfängen entwickelt es sich aus dem Amphibientypus heraus zu einem immer mächtiger werdenden Organe, an dem schon innerhalb der Reptilienreihe geordnete Rindenbildung vorhanden ist.

This above-quoted view of Edinger coincides by no means with the fact that the forebrain in embryonic selachians contains the main part of structures characteristic of higher vertebrates. Edinger's view thus is morphologically false, based as it is on a false interpretation of the selachian forebrain. Physiologically, Edinger's view perhaps may be right; but the principal reason for his view, the special connections of the 'episphaerium' in selachians, is not established by real observations; on the contrary, there are olfactory fibers of the second order present entering the 'episphaerium.'

Through the results of my observations on *Acanthias*, the opinion of Lundahl (v. Vetakad:s Handl., Bd. 59. No. 2, 1918), expressed in the most categorical manner, concerning the forebrain phylogeny has no real reason. Lundahl interprets the part of the brain dorsal to the *zonae limitantes* as a 'palaeopallium.' This view he bases on the suggestion that this part of the forebrain is built up by "numerous, little differentiated nerve-cells," diffusely arranged without forming typical layers. And he finds the statement of this description in papers of v. Kupffer, Kappers, C. L. Herrick, Edinger, Jakob, and Johnston. That Lundahl's opinion of the pallium in selachians is false follows immediately from the above description of the embryological development of the pallium in *Acanthias*.

This development also shows the extreme error of the interpretation of the phylogenetic development of the different cortices in the reptilian brain as given by different authors, according to whom the 'palaeopallium,' the 'archipallium,' and the 'neopallium' develop successively from a 'matrix' situated at the *fissura rhinalis externa*. Such a matrix does not exist in the ontogenetic development. The cortices develop simply from the underlying part of the neuroblastic layer and there are no signs present of a successive displacing of cortical units from a place of origin at the 'fissura rhinalis externa' to more medial situation. All that is true in such a description is that the cortices are developed successively, the pyriform cortex being developed a little before the general pallial cortex; but it is false that all cortices are derivatives from a common matrix, situated as said before. The hippo-

campal, the general pallial, and the pyriform cortices are differentiated parts of an earlier undifferentiated ancestral cortex, which may conveniently be called the primordial cortex. Such an undifferentiated cortex is perhaps that of cyclostomes. But in that case there is reason for the opinion that the cyclostomes are the only representatives of such a pallial type that have survived to our days.

The presence of the same cortical units in the selachians as in the higher vertebrates is a fact that throws a new and unexpected light upon the phylogenetic development of the forebrain in vertebrates, as will be pointed out further on.

HOLOCEPHALI

The forebrain of Chimaera

The forebrain of *Chimaera* has been regarded (Kappers, '08; Kappers and Theunissen, '07; Kappers and Carpenter, '11) as very important for the understanding of the everted forebrain in teleosts, as being partly inverted, partly everted. To this the objection may be made that in teleosts it is the pallium that is everted, in *Chimaera* subpallial parts, the stria medullaris. Thus I think that the forebrain of *Chimaera* has not the great importance for the understanding of the teleost brain as believed.

The forebrain of *Chimaera*, however, is very important for the understanding of forebrain morphology in lower vertebrates, especially in cyclostomes and selachians. There are conditions present which help us to understand the phylogenetic development of these brains and also of the brain in *Dipnoi*.

The exterior of the *Chimaera* brain is well known and it is enough to refer to the excellent figure given in the 'Vorlesungen' of Edinger. On this figure, however, the posterior part of the telencephalon is lettered 'Praethalamus,' a designation which, as Johnston has pointed out, is not right. The great length of the forebrain is due to the extreme prolongation of the postpallial parts of the subpallium which, as Schauinsland has shown in *Callorhynchus*, takes place in the ontogenetic evolution. The pallial parts are not subjected to such a prolongation process and

therefore exhibit more normal conditions. From the morphological point of view, this part of the forebrain affords a considerably greater interest than the caudal one.

The rostral part of the forebrain is inverted and rostrally evaginated.³ The bulbi olfactorii are inserted at the rostral end of each hemisphere. In the bulbi no bulbar ventricle is present.

The pallial parts are comparatively small, extending along the rostral part of the medial border of the forebrain. In the evaginated rostral part the pallium forms the main part of the medial wall of the lateral ventricle (fig. 24), but, proceeding toward the foramen monroi, the septal pallium gradually retires in the dorsal direction (figs. 25 to 28, *pall.*) and at the foramen monroi it is found almost quite dorsal to the ventricle (fig. 27). A medial zona limitans is very clearly pronounced, especially in the neighborhood of the foramen monroi. The lateral expansion of the pallium is not great. The lateral limit is marked by a distinct zona limitans lateralis (*z.l.l.*), lying a little below the upper corner of the lateral ventricle. Caudal to the foramen monroi the pallium is reduced to a dense cell-mass at the upper end of the transverse section (fig. 28). Here the pallium is no longer inverted as it passes over into the pallium-free 'praethalamus' of Edinger.

The pallial parts of the forebrain are inverted so as to bring the two pallia in contact with each other. In contradiction to the selachians, the pallia do not join in the mid-line, the holocephalians thus being more primitive in this respect. In *Chimaera* the two pallia are joined by an ependymal membrane, a tela, which in its rostral part forms a short and thin fold (figs. 27, 28, *ch.f.*) hanging down between the two hemispheres.

This chorioidal fold is of great interest as being the caudal continuation of an ependymal fold (figs. 25, 26, *ch.f.*) belonging to the upper wall of the recessus neuroporicus (*rec.n.*). This recessus in the young *Chimaera* brain is very well marked. From the lamina terminalis at the level of the foramen monroi, it extends

³ My description is based on transverse sections of a young *Chimaera* brain. In older specimens many of the structures described become less conspicuous.

forwards in the shape of a short cornet. This cornet is laterally compressed between the hemispheres. Its opening is very wide (fig. 26), situated just at the level of the foramen monroi. From near the tip of the cornet its dorsal wall is folded into a chorioidal fold (*ch.f.*) hanging down in the cornet and extending backwards in the epithelial roof of the forebrain as the medial chorioidal fold mentioned above. From the form and the height of the recessus neuroporicus it results that the ependymal tela of the forebrain in the pallial region is not fastened at the medial border of the inverted pallium (fig. 28), but some distance dorsal or lateral to this border, a point of attachment peculiar to Chimaera.

In the pallium there are two layers of nerve-cells present, a ventricular and a more distal one. The distal is not very distinct (figs. 24, 25, 27), mostly consisting of scattered cells, sometimes aggregated to form a sort of cell-lamina (fig. 24), sometimes coalescing with the ventricular layer (fig. 26). It is thus impossible to say whether there is really a regular cortical layer present; probably there is one, but studies on embryonic material are necessary for stating this. I have no such material.

The subpallial parts are very strongly developed. The lateral olfactory nucleus (*n.olf.l.*) is very large, covering most of the lateral surface of the forebrain. The lateral brain-wall is very thick and, a little before the level of the foramen monroi, projects a little into the ventricle, forming a swelling which perhaps may be considered as a 'corpus striatum' (*st.s.*). In this swelling is found a condensation of nerve-cells corresponding to the so-called 'epistriatum' in sharks. But this 'epistriatum' is subpallial, and thus not homologous with the 'epistriatum' in reptiles and birds, where it is pallial (hypopallium of Elliot Smith), situated dorsal to the zona limitans lateralis. Farther back from the foramen monroi the lateral wall of the ventricle exhibits another similar swelling (fig. 28, *st.s.2*) of very great distinctness. These two 'striatal' swellings surely are homologous with those of Acanthias. But as they have no special characteristics allying them with the 'striatum' of reptiles, I am not quite disposed to ascribe to them the value of a corpus striatum.⁴

⁴ Perhaps, however, they correspond to a real striatum, but there are no special reasons for accepting this view.

In the subpallial region of the forebrain the whole ventral part is covered by a distinct tuberculum olfactorium (*t.olf.*). Behind the pallial portion the tuberculum olfactorium ascends laterally on the brain as far as to the stria medullaris. Caudally, the tuberculum olfactorium ends at the posterior margin of the second 'striatal' swelling ('striatum' of Kappers and Carpenter), where the nucleus preopticus begins.

Dorsal to the medial border of the tuberculum olfactorium lies the medial septal nucleus (figs. 24 to 27, *n.m.s.*). This nucleus never enters the 'septal' portion of the evaginated forebrain. The nucleus lateralis septi is rudimentary. No cellular fimbrial portion of this nucleus is present. A 'sulcus septalis' is present (figs. 25, 26, *s.s.*).

Making exception of some special characters, such as the elongation of the telencephalon medium and the striatal swellings, the chimaeroid forebrain exhibits some conditions which place this type on a lowlier phylogenetic stage than the selachian, approaching that of the cyclostomes. Such a primitive character is that the hemispheres are not confluent with each other, but only joined by a chorioidal tela. Also the lack of a nucleated fimbrial portion of the subpallial septum is a primitive character.

DIPNOI

My sectioned material of dipnoan brains being not more than a frontal and a horizontal series of young *Protopterus annectens*, stained with iron-haematoxylin, I was not able to make any original studies on these animals, beyond the determination of the nuclei. I have found in *Protopterus* the same conditions as those already found by Elliot Smith in *Lepidosiren*.⁵ I have but

⁵ The forebrain of *Ceratodus* seems to be built up in principle in the same way as in *Protopterus* and *Lepidosiren*. In the last number of this Journal that has reached me (vol. 32, no. 4), however, Herrick has pointed out some very great differences between *Ceratodus* and the other dipnoans, but examining two transverse series of *Ceratodus* brains I failed to recognize these differences, quoted by Herrick from Bing and Burekhardt ('05). Herrick says: "The lateral outpouching . . . extends but little, if at all, rostrally of the terminal plate and there is no sagittal fissure separating two cerebral hemispheres behind the olfactory bulbs. The true (fully evaginated) cerebral hemisphere, accordingly, contains only the olfactory bulb." In my specimens there is a

little to add to his excellent description. I wish only to point out the fact that the pallial formation of *Protopterus* is still smaller than in *Lepidosiren*. The inversion of the pallium is not great, the medial borders of it do not come in contact with each other, but are widely separated, especially in the posterior part of the forebrain. A zona limitans medialis is present (figs. 29 to 34, *z.l.m.*). On the inside of this zone there is a sulcus limitans medialis (*s.l.m.*), occupying the dorsomedial corner of the lateral ventricle. The sulcus limitans hippocampi (pallii medialis) is conspicuous only in the posterior part of the forebrain a little before the foramen monroi (figs. 32, 33, *s.l.p.m.*). The zona limitans lateralis is obsolete, but a sulcus limitans externus is clearly seen. At the rostral end of the forebrain this sulcus joins the sulcus limitans medialis at the base of the bulbus olfactorius. In the pallium a cortical layer (primordial cortex) of scattered cells is present (figs. 29 to 34, *g.p.c.*). This layer passes uninterruptedly through the entire pallium. A very distinct medial swelling (as in *Lepidosiren*) of this cortex I have not found; yet there is an indistinct thickening at each side of the cortical layer. It is of great interest that the ventricular cell-layer at the upper end of the ventricle is very thick, protruding against the thinner part of the cortex (figs. 29 to 33, *g.p.c.*) between the two swellings. This is a feature tolerably well marked in the photographs of *Protopterus*, as well as of *Lepidosiren* (Elliot Smith), and reminds one of the general pallium rudiment in *Acanthias*.

The subpallial parts are extremely developed, as pointed out by Elliot Smith. In lateral view the subpallial parts occupy two-thirds of the height of the forebrain vesicle, and in medial view they cover almost the whole height. The ganglion cells, especially in the frontal part of the brain, are ventricular in position (fig.

sagittal fissure, especially well developed on the ventral side. On the dorsal side a sagittal fissure is also present, although of a peculiar nature. The hemispheres are very strongly evaginated, but the entire dorsal part of the medial wall of each hemisphere is made up by a strongly folded ependymal membrane, whose folds form a part of the 'lingula interolfactoria' of Bing and Burckhardt. Thus the forebrain is extremely evaginated, with the foramen monroi situated in the hind part of it. A fuller account of the remarkable brain of *Ceratodus* will be given later.

29), and it is here impossible to make a well-justified subdivision of these parts. But a closer examination shows that in the lateral brain-wall, basal to the sulcus limitans externus, there is a broad band of the brain-wall, where the ventricular layer is relatively thin (figs. 30 to 32, *n.olf.l.*), much thinner than in the basal part of the vesicle. This part corresponds to what Elliot Smith has called 'striatum.' Really this part (figs. 30 to 32) projects a little into the lateral ventricle so as to suggest a 'striatum,' but there are no special structures proving that it is really a striatum. I will call this part the lateral olfactory nucleus, although it corresponds probably to both this nucleus and a 'striatal' swelling in selachians.

The basal portion of the lateral ventricle is covered with a very thick ventricular cell-layer (figs. 29, 30, *t.olf.*). In the caudal third of the forebrain, the superficial portion of this layer is delaminated from the ventricle as to form a somewhat cortical, free cell-lamina encircling in transverse sections in the shape of a U the basal portion of the ventricle (figs. 31, 32, *t.olf.*). This cell-lamina is the cortex of the tuberculum olfactorium, as pointed out by Elliot Smith. This cortex, thus, is free from the ventricle in its caudal portion, but the rostral portion is always ventricular. Rostrally it is confluent with the ventricular cell-layer at the frontal pole of the brain.

The caudal margin of the tuberculum olfactorium cortex projects into a cellular process, representing a nucleus taeniae (figs. 33, 34, *n.t.*), so interpreted also by Elliot Smith. This process is pierced by small bundles belonging to the lateral forebrain bundle, as is also the rest of the tuberculum olfactorium cortex in this region of the brain.

The subpallial septum is divided into two parts, a rostral and a caudal. These are separated by a heavily ependymated sulcus septalis (figs. 29 to 33, *ss.*) as in selachians. This sulcus begins at the ventromedial corner of the ventricle about the middle of the forebrain (fig. 29) and goes from here obliquely upward to end at the foramen monroi a good way beneath the sulcus limitans medialis (fig. 33). The portion of the subpallial septum included in transverse sections between this sulcus and the sulcus septalis is

the fimbrial portion of the septum. This portion, the antero-dorsal, is filled up by more or less scattered cells derived probably from the ventricular wall of the fimbrial portion. These cells represent probably the nucleus lateralis septi. In the postero-ventral part of the septum the medial border of the tuberculum olfactorium is situated. This border is continued dorsad by a lamina of scattered small cell-groups, representing probably a nucleus medialis septi.

The dipnoan forebrain compared with the selachian

At first sight the forebrain of Protopterus seems not to have many features reminding one of that of Acanthias. Nevertheless, a closer examination will reveal dispositions recalling very strongly the selachian conditions. In the subpallial parts the great extension of the tuberculum olfactorium is common to the two groups. Also the extension of the lateral olfactory nucleus, as determined above, seems to be in principle the same, the apparent difference being caused by the greater differentiation in selachians. The septal portion of the subpallium is different, the fimbrial portion being much greater in Protopterus than in selachians. But there is, on the other hand, a great resemblance in conditions. A sulcus septalis occurs in both brains and the fimbrial portion does not accompany the medial border of the hippocampal formation dorsal to the foramen monroi as in higher vertebrates. In selachians, where the hemispheres are joined in the middle line, there is no tela fastened at the posterior margin of the fimbrial portion of the septum as in Protopterus, where the hemispheres are not joined. This tela in Protopterus is probably a part of the lamina (terminalis or) supraneuroporica, which has come to have its attachment at the fimbria owing to a secondary displacement of the nucleus lateralis septi, a displacement which is not difficult to follow in the form-series: Chimaera, Acanthias, Protopterus and Rana.

The pallial parts of the forebrain seem to be most difficult to compare in the dipnoan and the selachian brain. A comparison between the adult Acanthias brain and that of a Protopterus is

impossible to make, but using the brain of a 5-cm. *Acanthias* embryo, the points of comparison are not difficult to find out. In this embryo the cortex consists of two layers, a distal, where the cells are tangentially stretched, and a ventricular with perpendicular cells. The former corresponds to the cortical layer, the latter to the ventricular in Dipnoi. In selachians also the second layer delaminates from the ventricular position in the embryo, and there is formed a thick cortical plate which wanders up against the brain surface. This cellular mass has before been named 'the general pallial cortex.' To this cortex there is in *Protopterus* and also in *Lepidosiren* a corresponding formation in the dorsally greatly thickened cell-layer, bordering the ventricular ependyma dorsal to the ventricle.

Thus there is an agreement in brain structure in Dipnoi and Selachians—an agreement sufficiently great to allow the conclusion that the forebrain types of these groups are to be derived from the same ancestral type with inverted forebrain, with at least ventricular cortex or perhaps with an outer cortex and an inner ventricular cell-sheet. That the last alternative corresponds better with the facts follows from the statement that there are two layers present in *Petromyzon*. The double cortex, thus, is ancestral in the vertebrate series, and the common ancestors of Dipnoi and Selachii therefore might have had such an one.

THE EVERTED FOREBRAIN IN POLYPTERUS, GANOIDS, AND TELEOSTS

As I have before pointed out, the everted type of forebrain is derived from the inverted, and I have also given some special reasons for this opinion. But I think that no other reason is necessary than the fact that lowlier fish-types, as cyclostomes, selachians, holocephalians, and dipnoans are provided with an inverted forebrain.

As in these primitive fishes the pallium encloses two cell-layers, where a subdivision into three parts at least is indicated, it follows that these conditions may be traceable in the remaining fish-types: crossopterygians, ganoids and teleosts.

The principal characters of the subpallial parts are also the same in selachians, holcephalians, and dipnoans. Thus, these characters necessarily may be present also in the fish-types with everted forebrain, as these fishes belong to the same phylum.

In the following I will try to show that the everted forebrain also in details is built up like the inverted. I begin my description with *Polypterus*.

Polypterus bichir

The peculiar forebrain of this fish has not yet been described in detail. I therefore will try to make a fuller account of this forebrain.

The telencephalon in *Polypterus* is evaginated and everted. The evagination is represented chiefly by the ventricles of the bulbi olfactorii (fig. 35). These open into the medial ventricle just at the caudal end of the bulbi (fig. 36), where the foramen monroi thus should be situated. As will be pointed out later, the opening of the bulbar ventricles into the medial ventricle does not perfectly correspond to the foramen monroi in inverted brains.

The eversion of the forebrain is a very peculiar one (figs. 36 to 38). The dorsal part of the lateral wall of the forebrain or the pallium, as it will be named here, is of uniform thickness throughout its whole extent. This pallial lamella has a very great height, but a little above its middle it is bent double to the lateral side, in such a way that its morphological dorsal border points in a ventral direction. Through this eversion the epithelial roof of the forebrain is highly expanded to form a broad tela covering the whole forebrain as in teleosts. Under this everted pallial area lies the very strongly developed subpallial forebrain. The tela is medially strongly infolded, so as to form a longitudinal chorioidal fold (figs. 37, 38).

The pallium. Above I have called the everted portion of the forebrain vesicle the pallium. This requires a further explanation. I have before defined the pallium as being a brain part lying dorsal to the zonae limitantes and practically also dorsal to the sulci limitantes. As has been pointed out by Johnston, the bulbar

ventricle is continued into the lateral wall of the brain as the sulcus limitans externus, dorsal to which the pallium is situated. In *Polypterus* the bulbar ventricle also is continued backwards in the brain wall by a short sulcus (figs. 37, 38). This sulcus must be the sulcus limitans externus. Thus the brain parts dorsal to this sulcus may contain the pallium. Dorsal to the sulcus there follows in the rostral part of the forebrain in *Polypterus* a short ventricular band devoid of nerve cells (fig. 37, 38) covered by a simple thin layer of ependymal cells. Lateral to this band the brain wall is nearly without cells. In this way a distinct limit between the relatively cell-rich subpallial and cell-poor pallial parts is formed. This limit has not, except in the medial part, the character of a regular zona limitans (*z. l. l.*), owing to the fact that the cells in the pallium are situated at the ventricle, leaving the rest of it almost devoid of cells.

More caudally, where the sulcus limitans externus disappears, the cell-free ventricular space is filled up by small cells, and thus the ventricular part of the zona limitans also disappears. But here begins a deep sulcus, a *sulcus limitans pallii lateralis* (figs. 37, 38, *s.l.p.*), marking the ventral limit of the pallium.⁶ It lies exactly at the limit between the pallium and the subpallial parts and passes along the side of the forebrain almost to its caudal end. To some degree this sulcus depends upon the direction of the basal portion of the pallium, which is more or less inclined against the medial line. As in the caudal end of the forebrain the pallium becomes vertical, the sulcus becomes less pronounced.

In the pallium of *Polypterus* nerve cells are present in two different situations. The main part forms a relatively thick ventricular layer (figs. 35 to 38), the others are sparsely distributed in the dense neuropil mass of the pallium. Thus there are two pallial layers as in all hitherto described forms. The ventricular layer is subdivided into three parts (figs. 37, 38), a medial (*p.c.*), a dorsal (*g.p.c.*), and a lateral (*h.c.*). The medial part of the layer

⁶ In *Amia* Johnston has named this sulcus the 'sulcus limitans hippocampi.' In higher animals the 'sulcus limitans hippocampi' belongs to the medial wall of the inverted brain. It is not homologous to the ganoid one. Therefore, I have changed its name in *Polypterus*.

is relatively thick, with not very densely packed, relatively pale-colored cells. The lateral part contains densely packed, dark-colored cells and is thicker than the dorsal. Thus these subdivisions are histologically differentiated. As will be made more than probable, they represent cortical layers corresponding to the pyriform (*p.c.*), the general pallial (*g.p.c.*) and the hippocampal (*h.c.*) cortices in other vertebrates.

Subpallial parts. As already mentioned, the bulbi olfactorii contain a great bulbar ventricle, opening into the medial fore-brain with a foramen monroi-like opening. As this opening is bounded ventrally by subpallial parts, dorsally by pallial, it might perhaps be compared with the foramen monroi in other vertebrates. This would, however, be to commit a great error, the dorsal boundary being the morphological lateral part of the pallium, the pyriform lobe, and the dorsal boundary of the foramen monroi being in other vertebrates the medial part of the pallium, the hippocampal lobe. The true foramen monroi thus should lie between the subpallial boundary of the opening and the external border of the everted pallium.

The bulbar opening is, as said above, ventrally limited by a subpallial part. This at the foramen forms a small subpallial septum (fig. 36, *sp.*). In this septum enters a process of the nucleus olfactorius anterior (*p.n.o.a.*) or lobus olfactorius. The cells in this process stain somewhat darker than those of the anterior olfactory nucleus. The process comes from the ventromedial part of the granule-cell layer (fig. 35, *p.n.o.a.*) and corresponds perfectly to the process of the same nucleus described by the present writer in *Osmerus*. The position of this process in the septum of *Polypterus* makes it probable that it represents a septal nucleus, the nucleus medialis septi in other vertebrates, this nucleus being many times a backward continuation of the nucleus olfactorius anterior or lobus olfactorius.

As in other fishes, the precommissural body is present in *Polypterus*. It is subdivided into a pars precommissuralis superior (figs. 37, 38, *p.p.s.*) and inferior (*p.p.i.*), a pars commissuralis, and a pars postcommissuralis or nucleus preopticus.

The superior part of the precommissural body in *Polypterus* is very strongly developed, forming a great cell-mass extending longitudinally from the caudal margin of the bulbus olfactorius to the commissural bed. Transversely it occupies the space between the sulcus limitans pallii and the lower vertical part of the ventricle. The sulcus limitans externus thus passes along the middle of the pars superior. Laterally, the rostral portion of the pars superior extends toward the lateral surface of the brain. The caudal part is more ventricular, with fewer cells.

In the pars superior a subdivision is indicated by the cell formation at the ventricular lumen. Above it has been mentioned that there is a cell-free space present just under the pallium in the rostral end of the brain. More caudally this space is enclosed between the sulcus limitans pallii and the sulcus limitans externus. At this point rows of small deeply staining cells make their appearance and soon occupy the upper half of the superior part. This represents the nucleus olfactorius lateralis. Under this region the cells are more scattered, paler.

At the rostral end of the brain the dorsal subdivision of the pars superior is well separated from the pars inferior, more caudal the two nuclei join more intimately. The pars inferior, however, is always distinguishable, occupying its position at the wall of the medial ventricle.

The precommissural body forms the commissural bed. This part of the nucleus is the pars commissuralis.

Behind the commissura anterior complex the nucleus preopticus begins, dorsally limited by the sulcus limitans pallii lateralis and the pallium.

In all parts of the precommissural body the main part of cells are more or less densely grouped along the ventricular wall. In the outer portions, however, there are scattered cells present. These scattered cells are arranged ventrolaterally from the commissural bed in a diffuse layer through which passes a lateral pallial tract on its way to the commissure. In the caudal prolongation of this layer lies a great nucleus entopeduncularis or taeniae (Johnston, fig. 41, *n.t.*), penetrated by the lateral forebrain bundle.

The above description is based upon a transverse series of sections through an adult *Polypterus* forebrain. But I have also studied the forebrain of younger stages. Through the kindness of Professor Jägerskiöld (Gothenbourg), I had the opportunity of investigating two stages, one (with retained outer gills) of 4.5 cm. total length and the other of 12.5-cm. These stages exhibit in principle the same morphological arrangement in the forebrain as the adult. In the 4.5-cm. stage the lateral olfactory nucleus is under formation from the pars superior of the precommissural body, as suggested in the adult. The eversion of the pallium is in principle the same as in the adult, but the bending of the upper portion of the pallium is in the youngest stage by far not so advanced as in the 12.5-cm. stage and in this it is much less advanced than in the adult. Of very great phylogenetic interest is the fact that the descending part of the pallium is much greater in the adult than in the 12.5-cm. stage and that in the 4.5-cm. stage one can scarcely speak of a descending portion, the pallium being composed of a high ascending part and a bent-out portion of smaller size. In the larger stages the brain-case is very spacious dorsoventrally and there is a rather wide free space between the roof of the brain case and the pallium. In the 4.5-cm. stage, however, the pallium lies in the closest contact with the roof of the skull and the relations between skull and forebrain are such as to make it evident that the bending out of the pallium is caused by lack of space for the outgrowing of the pallium in the dorsal direction. This stage of 4.5-cm. makes it very probable that it has been preceded by a stage where the pallium was uneverted, directed straight dorsad or perhaps bent in a little. The latter presumption is based on the fact that the pallium at the line of attachment of the tela makes a little bending in opposite direction to the general bending of the pallium. Thus, I find it very probable that in younger *Polypterus* stages there is even a somewhat inverted forebrain present. This being the case, the forebrain of *Polypterus* may serve very well for connecting the everted forebrain type with the inverted as present in dipnoi or holocephalians. The chief difference is the lack of forward evagination in *Polypterus*, but in *Chimaera* this evagination is very small, as pointed out before.

Ganoids and teleosts

In *Polypterus* all structures characteristic of all other fishes are present. In ganoids and teleosts these structures are repeated, but somewhat modified. Thus it is not necessary to describe minutely the brain of those fishes. I can restrict myself to the most striking differences.

In all ganoids the pallium is not of uniform thickness throughout, as in *Polypterus*, and thus the outer surface is not parallel with the ventricular. In *Acipenser* the ventricular surface is transversely rather convex, but the outer surface is flat. Thus, in a transverse section the distance between the zona limitans and the point of attachment of the tela is much longer than the distance between the latter point and the fovea externa. In other ganoids this difference in length is much greater, as the pallium is much thicker in the middle than in *Acipenser*, and in teleosts the tela is attached close to the fovea externa.

Thus, in actinopterygians (ganoids and teleosts) the development seems to go from a moderately thickened pallium to an extremely thickened one; that is, from a pallium with relatively long lateral outline to one with very short.

In all actinopterygians the pallial cells are grouped in three more or less well-limited parts, corresponding to the three parts in *Polypterus*. But in all actinopterygians the pallial cells have wandered in from the ventricular sheet to a greater extent than in *Polypterus*. In *Acipenser*, *Scaphirhynchus*, and *Polyodon* the cortical cells form cell-laminae with cortical arrangement throughout the whole pallium, with but few cells in the ventricular position. In a young *Lepidosteus* (fig. 39) a ventricular layer covers the whole ventricular surface, but the main part of the pallial cells has wandered in.

In teleosts the three pallial parts are very well limited, but the cortical arrangement of cells is not always well pronounced. In *Osmerus* I have described this arrangement (see my paper in *Acta Zoologica*, vol. 1, 1920).

The zona limitans is very conspicuous in *Lepidosteus* (fig. 39, *z.l.l.*) and *Amia* (Johnston) and in many bony fishes, but in

Acipenser, *Scaphirhynchus*, and *Polyodon* the zona is only to be seen in the foremost part of the forebrain. There is, however, no difficulty in making out the limit between the pallial and subpallial parts, owing to very pronounced structural differences between these parts, the pallial neuropil being denser than the subpallial.

A sulcus limitans pallii lateralis generally is not present in ganoids and teleosts, except in *Amia*, where Johnston has found such a sulcus, and in *Acipenser*, where it is present in the foremost part of the forebrain.

The subpallial parts in ganoids in principle are built up as in *Polypterus*. In *Lepidosteus*, *Amia*, and teleosts a very well-defined nucleus olfactorius lateralis below the zona limitans is present. In *Lepidosteus* (and *Amia*) this nucleus is very interesting, being formed by cells grouped together in islets or clusters, very well differentiated in the preparations from all other cells in the subpallial parts. Thus, it is here possible to make out the limits of this nucleus more fully than in other fishes. The nucleus olfactorius lateralis is composed of a lateral part, forming a cell-lamina in the lateral portion of the brain-wall (fig. 39, *n.olf.l.*). From this lamina a process extends medially to join the ventricular ependymal layer just below the zona limitans, dorsal to the precommissural body (*p.p.s.*). Through this process the ventricular situation of the nucleus olfactorius lateralis is clearly demonstrated in *Lepidosteus*. Johnston has shown that the nucleus olfactorius lateralis in *Amia* is built up almost in the same way. The opening of the bulbar ventricle is caudally continued by a groove of different shape and length in different fishes. In *Acipenser* this groove is not very pronounced, forming a shallow concavity in the foremost part of the forebrain. In *Lepidosteus* the groove is very broad, rostrally forming a semicircular excavation (fig. 39, *s.l.l.*) in the ventricular wall. Caudally, near the commissural bed, the excavation becomes shallower and disappears. The ependymal layer, covering the excavation, is thick. In *Amia* (Johnston) the sulcus is a narrow ependymal groove. In *Osmerus* (teleost) the rostral part is a relatively broad excavation, as in *Lepidosteus*. Caudally this excavation becomes

narrower and disappears, leaving an ependymal thickening as witness to its earlier existence also in the caudal region of the forebrain.

In all fishes the precommissural body is divided into an upper and a lower portion: pars superior and pars inferior. A nucleus olfactorius anterior pars precommissuralis I have found in *Osmerus* in the same position as in *Polypterus*, but extending further backwards. In ganoids I have found no such nucleus, owing probably to the preservation of my material. In *Polypterus*, as in all ganoids and teleosts, the preoptic nucleus is composed of a magnocellular and a parvocellular part. Also a pars recessi is present. The recessus preopticus is very broad in ganoids, narrower in teleosts.

The nucleus taeniae (entopendularis) in ganoids and teleosts, as in *Polypterus*, is penetrated by the lateral forebrain bundle.

Summary of the forebrain in Polypterus, ganoids, and teleosts

The forebrain in all fishes is built up according to a common scheme, but *Polypterus*, in the character of the eversion of the pallium and in the broad medial ventricle, occupies a separate place. The shape of the pallium should perhaps be taken as argument for an idea of a special eversion process, independent of that in other fishes. The fact, however, that the subpallial parts do not differ from those in other fishes seems to overthrow such a theory. Thus, I think that the everted forebrain in *Polypterus* is to be derived from the same source as that in other fishes with everted forebrain. *Polypterus*, thus, in this respect belongs more to the actinopterygian stem than to the crossopterygean-dipnoan.

The scheme of forebrain construction in all fishes with everted forebrain thus may be that of the diagram, figure 40. In this diagram all nuclei are brought back to the probable position of their matrix formations at the ventricular wall. Beginning from the dorsal border of the pallium and following the inside of the ventricle ventrad, we meet the following structures:

- I. Pallial parts.
 - a. Hippocampal pallium (*h.p.*).
 - b. General pallium (*g.p.*).
 - c. Pyriform pallium (*p.p.*).
- II. Zona limitans (lateralis) (*z.l.l.*) and sulcus limitans pallii lateralis (*s.l.p.l.*).
- III. Subpallial parts.
 - d. Nucleus olfactorius lateralis (*n.olf.l.*) and sulcus limitans lateralis (*s.l.l.*).
 - e. Corpus precommissurale, pars superior (*p.p.s.*).
 - f. Nucleus olfactorius anterior pars precommissuralis (*p.n.o.a.*).
 - g. Nucleus entopeduncularis, or taeniae (*n.t.*).
 - h. Corpus precommissurale pars inferior (*p.p.i.*) and, in the caudal part of the forebrain, nucleus preopticus.

COMPARISON BETWEEN THE EVERTED AND THE INVERTED
FOREBRAIN IN LOWER VERTEBRATES

A diagram of the inverted forebrain made up in the same way as that of the everted shows the following structures (fig. 41):

- I. Pallial parts.
 - a. Hippocampal pallium (*h.p.*).
 - b. General pallium (*g.p.*).
 - c. Pyriform pallium (*p.p.*).
- II. Zona limitans (*z.l.*)
- III. Subpallial parts.
 - d. Nucleus olfactorius lateralis (*n.olf.l.*) and sulcus limitans lateralis (*s.l.l.*).
 - e. Tuberculum olfactorium (*t.olf.*).
 - f. Nucleus medialis septi (*n.m.s.*).
 - g. Nucleus entopeduncularis, or taeniae (Dipnoi) (*n.t.*).
 - h. Nucleus lateralis septi (*n.l.s.*) and, in the caudal part of the forebrain, nucleus preopticus.

The idea of the comparative anatomy of the everted forebrain recently has undergone a very important change, especially through the excellent works of Johnston. This author is of the opinion that the so-called 'striatum' of earlier authors belongs to the pallium. He names this part, situated dorsal to the zona limitans, the primordium hippocampi. In this part Kappers and Sheldon have found three different parts, separated in teleosts by well-marked sulci. These parts, however, have been differently interpreted. Kappers supposes that they represent a lateral palaeopallium, a dorsal striatum, and a medial

epistriatum—a conception which does not coincide with the fact that this epistriatum is situated morphologically ventral to his striatum. Sheldon is of the opinion that the three parts are: a medial primordium hippocampi, a lateral nucleus olfactorius lateralis with a pyriform lobe, and a centrally situated palaeostriatum. This opinion is contradicted by the fact that his primordium hippocampi is morphologically ventral to his nucleus olfactorius lateralis and to his pyriform lobe.

In my paper on the forebrain and 'tweenbrain of teleosts I have adopted another terminology. The primordium hippocampi of Johnston I have named 'primordium pallii.' Now I will change this name to 'pallium' only, as being more consistent with the conditions in lower vertebrates. This pallium corresponds fully with the pallium in inverted and evaginated brains as being a part of the brain situated dorsal to the zona limitans and the sulcus limitans externus. This pallium is subdivided into three parts, corresponding to the parts named by Kappers in another way. I have called them, respectively, the primordium hippocampi, the general pallium, and the pyriform lobe, and this nomenclature seems to me to be a consequence of my idea of the pallium in teleosts. In inverted brains the most dorsal (=medial) part of the pallium is the hippocampal pallium, the middle (=dorsolateral) part the general pallium, and the ventral (=lateral) part the lobus pyriformis. Under the supposition that the subdivision of the pallium is the same in all lower vertebrates, the homology must be well settled. This supposition is no arbitrary one, but is supported by the fact that it is present already in selachians and Polypterus, that is, in more primitive forms than ganoids and teleosts. Thus, I think, there is no serious objection to make against the homology of the pallial parts. It is clear, however, that the homology is not perfectly proved until it is shown that the fiber connections do not speak decidedly against the homology. But it is still too early to draw the fiber connections into the discussion, these being too little known in selachians and holoccephalians and practically unknown in Dipnoi and Polypterus. But so far as known to-day, the fiber connections do not speak against the homology.

In the subpallial parts the nucleus olfactorius lateralis in all brains, everted as well as inverted, occupies the same position in the lateral part of the brain-wall at the sulcus limitans externus, just ventral to the zona limitans, and the conditions especially in *Lepidosteus* make the homology of that nucleus very probable. Also the fact that a part of the lateral olfactory tract splits up in this nucleus both in inverted and everted brains is of the nature to settle the homology.

In the inverted brain the tuberculum olfactorium is formed from the neuroblastic layer ventral to the nucleus olfactorius lateralis or the sulcus limitans lateralis (externus). In the everted forebrain the superior part of the nucleus precommissuralis occupies the corresponding part of the ventral wall, and therefore must be homologous with the tuberculum olfactorium. That there must be a homologue of the tuberculum olfactorium present in ganoids and teleosts follows from the fact that this part of the brain is present in selachians, holocephalians, Dipnoi and tetrapods, and in ganoids and teleosts there is no other part to be found with corresponding position. The difference between the true tuberculum olfactorium and that of ganoids and teleosts is that in the latter groups the cells do not form a cortical layer as in the former, but keep their original place at the ventricular wall. It is, however, possible that the ventricular position is secondary in ganoids and teleosts, as well as in Dipnoi and Amphibia!

Ventral to the corpus precommissurale pars superior in ganoids and teleosts comes the pars inferior of the same corpus. This nucleus is ventricular and forms the ventral part of the brain-wall rostral to the commissural bed. Its morphological position is the same as that of the nucleus lateralis septi of the inverted brain, since it must be borne in mind that the subpallial septum is the medial ventral part of the forebrain ventricle which is brought into its septal position through the evagination of the hemispheres. Thus the morphological situation of the pars inferior of the pre-commissural body is that of the nucleus lateralis septi in the inverted forebrain.

As to the homology of the nucleus medialis septi in the inverted and nucleus olfactorius anterior pars precommissuralis in the everted forebrain, some facts seem to support this view:

1. In *Polypterus*, where there is a small septum present, the nucleus olfactorius anterior (= lobus olfactorius) sends a process (pars precommissuralis) into this septal structure.

2. In *Acanthias*, where in front the nucleus olfactorius lateralis occupies the ventral part of the frontal surface of the hemispheres, it sends a cell-lamina into the subpallial septum. This cell-lamina is the nucleus medialis septi.

3. In the embryo of *Rana* (according to investigations made at this institute by Miss G. Söderberg) the granule cell-zone in front of the hemispheres is continued into the septum by the nucleus medialis septi.

The nucleus taeniae, or entopeduncularis, in teleosts is probably a derivation from the nucleus precommissuralis pars superior. Certain observations in the ontogeny of *Salmo* (made at this institute by G. Lindén) have shown its derivation from the portion of the ventricular wall occupied later by the superior part of the precommissural body. In *Dipnoi*, where a nucleus taeniae is also present, it is associated with the tuberculum olfactorium. As well in ganoids and teleosts as in *Dipnoi*, it is penetrated by the lateral forebrain bundle. I think there may be no serious objection to be made against this homology.

Hitherto, I have thought, like Johnston, that the nucleus taeniae is a homologue of the 'somatic area' in selachians. This view I do not now find well grounded, as my investigations on the selachian brain seem to have shown that the 'somatic area' in selachians is part of an unusually large nucleus preopticus.

After the above discussion I venture to make up the following scheme of the forebrain homologies in lower vertebrates:

		INVERTED FOREBRAIN	EVERTED FOREBRAIN
Pallial parts	1	Hippocampal pallium	Lateral part of the everted pallium
	2	General pallium	Dorsal part of the everted pallium
	3	Pyriform pallium with hypopallium	Medial part of the everted pallium
Boundary lines	4	Absent (generally)	Sulcus limitans pallii (sometimes present)
	5	Zona limitans lateralis	Zona limitans
	6	Zona limitans medialis	Absent
Sub-pallial parts	7	Sulcus limitans externus	Sulcus limitans externus
	8	Sulcus limitans medialis	Absent
	9	Nucleus olfactorius lateralis	Nucleus olfactorius lateralis
	10	Tuberculum olfactorium	Nucleus precommissuralis pars superior
	11	Nucleus taeniae	Nucleus entopeduncularis or taeniae
	12	Nucleus lateralis septi	Nucleus precommissuralis pars inferior
	13	Nucleus medialis septi	Nucl. olfactorius anterior pars precommissuralis

THE EVOLUTION OF THE FOREBRAIN IN THE LIGHT OF VERTEBRATE PHYLOGENY

The evolution of the forebrain in vertebrates is a problem which cannot be solved by the comparative anatomy of the brains of now living animals only, as those represent only the upper terminal branches of the great vertebrate stem. Certainly, however, every worker in comparative neurology has formed for himself an idea about this problem, which coincides with his idea about brain morphology. Very few, however, have tried to examine to what degree their opinions answer to the vertebrate phylogeny, established on the palaeontological results or on characters taken from other organic systems. It is clear that a phylogeny of the forebrain, if a well-grounded one, must on the whole correspond with the phylogeny established on the anatomy of other organs.

The skeleton is an organic system of the greatest importance for vertebrate phylogeny, as it can be studied not only in living, but also in extinct animals, and our idea concerning vertebrate phylogeny is thus grounded chiefly on the comparative anatomy of this system.

The living species of the groups of vertebrates represent the upper tips of the widely branched vertebrate phylum, and thus they are not to be derived from each other. In the same way it is impossible to derive the brain structures of one of these groups from those of another. This is a rule against which comparative neurology has sinned very much. Many times the brain of a Petromyzon, a shark, a teleost, an urodele, a frog, a reptile, a bird, and a mammal have been combined together as a typological series which has served also for demonstrating the phylogenetic process. The ground for the phylogeny of the brain structures must be the genealogic tree, constructed on the knowledge of the extinct animals also. Such a genealogic tree has many times been built up, and it might in broad lines have the aspect of the diagram, figure 42.

In this diagram stem-groups, as crossopterygians, stegocephalians, cotylosaurians, are marked with circles and the groups of living vertebrates, probably derived from these directly or indirectly, are drawn up from the periphery of the circles. This is done in order to avoid mistakes in the closer arrangement of the branches in relation to each other. The full-drawn lines show the chronological distribution of the groups, based upon palaeontological discoveries. In this diagram there are two hypothetical ancestral groups lettered *x* and *y*. The *x*-form represents the common ancestor to the cyclostomes and all other vertebrates, the *y*-form the common ancestor of selachians-holocephalians and other vertebrates.

The forebrain of extinct crossopterygians

It is a very delicate affair to try to get an idea of the brain of an extinct animal group, and I wish to accentuate that what here will be said has more the character of a keen experiment of thought than a well-grounded theory. But in the light of the structure of

the brain in the living descendants, it seems possible to get a rough idea of this subject, especially if the phylogenetic relations of the extinct groups are used for guidance.

From the crossopterygians the Dipnoi, the polypterids, and the actinopterygians are generally derived. Of the crossopterygian descendants, the Dipnoi have an inverted forebrain, the polypterids and actinopterygians an everted. Assuming that the descent of these three groups of fishes is rightly interpreted by paleontologists, the eversion took place in the crossopterygian group or in nearly allied descendant groups. The crossopterygians are found from the lower Devonian to the upper Cretaceous. But the actinopterygians began also in the Devonian with crossopterygian-like fishes, as the palaeoniscids and others. Thus the eversion of the forebrain may have taken place very early, probably already in Devonian fishes, or perhaps earlier.⁷

From primitive crossopterygians the Dipnoi undoubtedly are descendants. The earliest Dipnoi are found in the Devonian. In the living species the forebrain is inverted. Thus it seems to be probable that the inverted forebrain, as being the primitive type of vertebrate forebrain, also was the brain type in primitive crossopterygians of the Devonian. As in all recent descendants of the crossopterygian group and also in the plagiostomes the pallium contains two cell-layers, a cortical and a ventricular, this must also have been the case in the crossopterygians. Further, it is probable that the pallium was already subdivided into the typical three parts, the hippocampal, the general, and the pyri-form pallium. This is a conclusion drawn from the fact that in Polypterus, ganoids and teleosts the subdivided pallium is the rule. But also in Dipnoi there are signs of a subdivided pallium. Also in the parallel group of selachians a similar subdivision is present, at least in the embryo. The eversion of the forebrain in Polypterus and ganoids must have taken its origin from a slightly

⁷ Judging from the shape of the skull and the position of the optic and trochlearis foramen in the newly described crossopterygian genus *Wimania* from the Triassic, the forebrain ought to have been very elongate and laterally compressed as in dipnoans and Polypterus. In the triassic palaeoniscid *Birgeria* the brain-case is much shorter, laterally compressed and rapidly narrowed in front, suggesting a forebrain of the same type as in the young *Lepidosteus*.

inverted forebrain, where the medial portions were not joined in the mid-line. Such a forebrain is present in the recent Dipnoi. Thus it seems very probable that also in primitive crossopterygians such an inversion was present. This assumption is supported by the fact that in the holocephalians and in petromyzonts this brain type is the rule.

In the ependymal roof of the forebrain there is in ganoids and teleosts and also in Dipnoi and holocephalians a medial infolding in front of the paraphysis. This medial fold might have been present also in the primitive crossopterygians. (In holocephalians this fold begins at the top of the recessus neuroporicus.) A paraphysis was also probably present.

The hemispheres were in primitive crossopterygians probably moderately evaginated. This is an assumption based upon the fact that in Polypterus, ganoids, and teleosts the evagination is confined to the foremost part of the forebrain, the bulbus olfactorius, and in holocephalians also is not very pronounced. Against this assumption is the fact that in recent Dipnoi the hemispheres are excessively evaginated. But here the evagination is of a quite peculiar nature, being confined to a great extent to subpallial brain parts. The evagination, corresponding to that in ganoids, that is, the evagination that ends in the bulbus, is not greater, however, than in holocephalians. In recent Dipnoi (except in *Ceratodus*) the bulbar ventricle is rudimentary. As a special bulbar ventricle is absent in holocephalians, present in Polypterus and ganoids and also in selachians and petromyzonts, it seems probable that also in primitive crossopterygians there was a bulbar ventricle, at least of moderate size.

In all vertebrates with inverted and evaginated forebrain a zona and a sulcus limitans medialis (except in cyclostomes) and a sulcus limitans externus occur. These structures might have also been present in primitive crossopterygians. As the occurrence of a zona limitans lateralis is variable in vertebrates, it is not possible to determine whether such a zona was present in the crossopterygians.

In the subpallial parts, the ventricular position of the nuclei in Dipnoi seems to indicate that the same was the case in the

Crossopterygii. But, on the other hand, it must be borne in mind that the nucleus olfactorius lateralis and the nucleus taeniae in ganoids and teleosts are free from the ventricular ependyma. In Dipnoi the nucleus taeniae is also removed from the ependyma and the tuberculum olfactorium in its caudal part has wandered in from the ventricular wall. Further, the tuberculum olfactorium in selachians and holocephalians, as well as in reptiles and mammals, is removed from the ependyma. Therefore, I am disposed to believe that the primitive crossopterygians had a tuberculum olfactorium removed from the ventricular wall.

In summary, this hypothetical crossopterygian forebrain resembles the holocephalian, if the great secondary prolongation of the telencephalon medium of the latter and the subdivision of the pallium of the former are disregarded.

The forebrain of the common ancestors of selachians and holocephalians

The holocephalians probably are a very old group of fishes. The earliest representatives are found in Jurassic layers, but the group might have been much older. The holocephalians undoubtedly are related to the selachians and the holocephalian stem is probably a very early branch from the selachian. Selachians are known from the Lower Devonian period.

Assuming that the holocephalians and selachians are descendants from a common ancestral vertebrate type, the forebrain of the type was an inverted and evaginated one. In selachians the inversion is very pronounced, and the halves of the pallium are joined in the mid-line almost throughout their whole length. This is a condition that must be regarded as due to secondary fusion. The evaginated frontal parts or the forebrain also are secondarily joined to form the massive septum. In seeking the ancestral selachian type of forebrain, these secondary characters must be removed. Taking away these characters, the selachian forebrain will be reduced to a more or less holocephalian type with lateral bulbi olfactorii and subdivided pallium.

The lateral position of the bulbi probably is primitive. This assumption is based on the fact that the bulbar formation in the

embryo of *Petromyzon*, selachians, Dipnoi, ganoids, teleosts, and amphibians is formed at the lateral surface of the forebrain vesicle and from this position brought to its frontal position.

The forebrain of the common ancestors of the selacho-holocephalians and the crossopterygians (hypothetical form y)

Comparing the schemes of the brain types of selacho-holocephalians and primitive crossopterygians, we find them to cover each other almost perfectly, the former being, however, more primitive in the supposed lateral position of the bulbi olfactorii.

From this agreement the conclusion may perhaps be permitted that the common ancestors of the selachians and primitive crossopterygians had a forebrain built up more conforming to the selacho-holocephalian ancestral type than to the crossopterygian.

The forebrain of the common ancestors of gnathostomian fishes and cyclostomes (hypothetical form x)

The cyclostomes represent a very old vertebrate group. The position of the Devonian genera *Palaeospondylus* and *Heterospondylus* being very uncertain, no unquestionable extinct species are hitherto known. The morphology of the living genera, however, indicates that these are in many respects reduced forms. The absence of paired limbs seems to connect the cyclostomes with those Silurian fish-types included in the class of anaspids (*Lasanius*, *Birkenia*, etc.). Perhaps the living cyclostomes could be eel-shaped semiparasitic descendants from such fish-types.

The evagination of the forebrain in *Petromyzon* probably is reduced together with the reduction of the lateral ventricles, as has been explained before, but there are, on the other hand, no signs present indicating that the frontal evagination has ever been more than a bulbar one. The septum, if regarded as present, is built up only by cells belonging to the granular layer of the bulbus olfactorius. The pallium thus is excluded from the 'septum.'

In the pallium there are no signs indicating a subdivision. Also in the subpallial parts no special nuclei are indicated.

The presence of a sulcus limitans externus undoubtedly is a primitive character.

The absence of a medial infolding of the tela and of a zona limitans lateralis may be primitive or secondary; it is not possible to decide which of these alternatives is right.

The situation of the bulbi olfactorii in front probably is secondary. The entrance of the olfactory nerves somewhat laterally and the lateral position of the bulbar formation in the embryo seem to indicate this.

The preceding attempts to deduce the type of the forebrain in the hypothetical ancestral types are very uncertain, but the uncertainty increases very much in making an attempt to reconstruct the brain type of the common ancestors of the cyclostomes and the other fishes. But it must be borne in mind that the only way to get any information of these brains is that based on deduction from the recent fauna.

The brain type, however, of the common fish ancestors may have been inverted and at least evaginated in the bulbus olfactorius, which had a lateral position. The pallium was probably not subdivided. There was a sulcus limitans lateralis present. The subpallial nuclei were not differentiated.

According to the ideas set forth above, the subdivision of the pallium has taken place in a very remote period, probably already in the Silurian. In this period also the subpallial nuclei seem to have been differentiated and the frontal evagination taken place. The inversion of the forebrain is primitive and the Silurian fishes probably had an inverted and also evaginated forebrain. The eversion, characteristic of *Polypterus*, ganoids, and teleosts, probably took place in the early Devonian period.

PLATES

ABBREVIATIONS

<i>b.o.</i> , <i>Bulb.olf.</i> , bulbus olfactorius	<i>p.h.</i> , primordium hippocampi
<i>ch.f.</i> , chorioidal fold of the recessus neuroporicus	<i>p.n.o.a.</i> , nucleus olfactorius anterior pars precommissuralis
<i>Cort. 1.</i> , outer cortical layer	<i>p.p.</i> , pyriform pallium
<i>Cort. 2.</i> , inner cortical layer	<i>p.p.i.</i> , corpus precommissurale pars inferior
" <i>Ep.</i> ," epistriatum of Edinger	<i>p.p.s.</i> , corpus precommissurale pars superior
<i>E.t.</i> , eminentia thalami	<i>prim.c.</i> , primordial cortex
<i>f.</i> , fimbrial portion of septum	<i>r.n.</i> , recessus neuroporicus
<i>f.ext.</i> , <i>fov.e.</i> , fovea externa	<i>sc.c.</i> , scattered cells of the primordial cortex
<i>G.h.</i> , ganglion habenulae	<i>s.l.e.</i> , sulcus limitans externus
<i>g.p.</i> , general pallium	<i>s.l.i.</i> , sulcus limitans internus (medialis)
<i>g.p.c.</i> , general pallial cortex	<i>s.l.l.</i> , sulcus limitans lateralis
<i>g.p.t.</i> , general pallial thickening	<i>s.l.p.</i> , <i>s.l.p.l.</i> , sulcus limitans pallii lateralis
<i>h.c.</i> , hippocampal cortex	<i>s.l.p.m.</i> , sulcus limitans pallii medialis
<i>h.em.</i> , hippocampal emigrating cells	<i>Sp.</i> , septum
<i>h.p.</i> , hippocampal pallium	<i>s.s.</i> , sulcus septalis
<i>H.v.</i> , hemispherical ventricle	<i>st.s.</i> , striatal swelling
<i>n.l.s.</i> , nucleus lateralis septi	<i>st.s.2.</i> , second striatal swelling
<i>n.m.s.</i> , nucleus medialis septi	<i>sub.</i> , 'subiculum'
<i>n.m.s.v.</i> , nucleus medialis septi pars ventralis	<i>t.olf.</i> , tuberculum olfactorium
<i>n.olf.l.</i> , nucleus olfactorius lateralis	<i>z.l.l.</i> , zona limitans lateralis
<i>n.preopt.</i> , nucleus preopticus	<i>z.l.m.</i> , zona limitans medialis
<i>n.t.</i> , nucleus taeniae	
<i>o.v.</i> , olfactory ventricle	
<i>pall.</i> , pallium	
<i>p.c.</i> , pyriform cortex	

PLATE 1

EXPLANATION OF FIGURES

1 *Ammocoetes*, 1.5 cm. Transverse section through the posterior part of the forebrain.

2 *Ammocoetes*, 3 cm. Transverse section through the opening of the caudal ventricular diverticulum.

3, A, B Transverse sections, A, through the 'primordium hippocampi' of *Petromyzon*, B, through the 'eminencia thalami' of *Triton*.

4 *Ammocoetes*, 3 cm. Transverse section through the opening of the olfactory ventricle.

5 *Acanthias*, 3.3 cm. Transverse section through the foramen monroi.

6 *Acanthias*, 3.3 cm. Transverse section through the middle of the forebrain. Same series as figure 5.

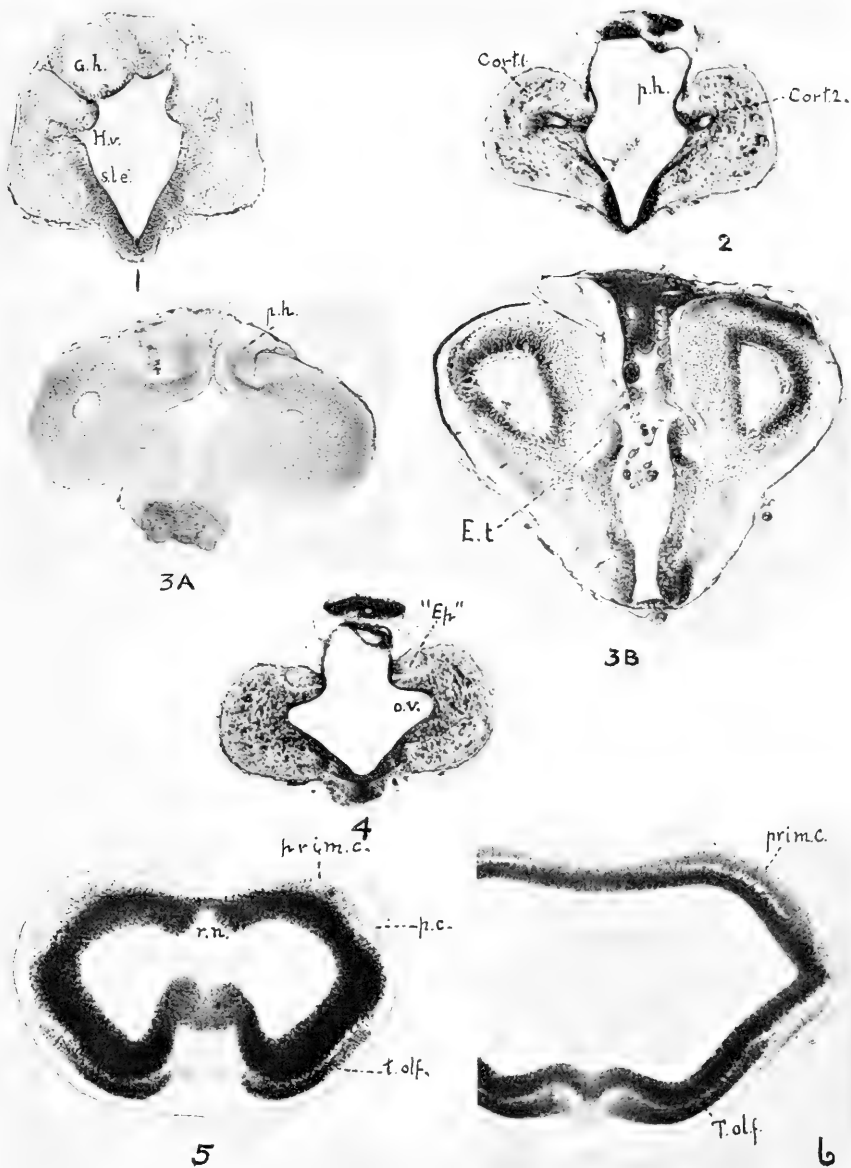


PLATE 2

EXPLANATION OF FIGURES

7 *Acanthias* 3.7 cm. Transverse section, passing through the bulbus olfactorius.

8 *Acanthias*, 3.7 cm. Transverse section through the lateral part of the pallium.

9 *Acanthias*, 4 cm. Transverse section taken before the foramen monroi.

10 *Acanthias*, 4 cm. Transverse section a little behind the front part of the foramen monroi.

11 *Acanthias*, 5 cm. Transverse section taken through the rostral part of the hemispheres.

12 *Acanthias*, 5 cm. Transverse section taken somewhat behind the section figured in figure 11.

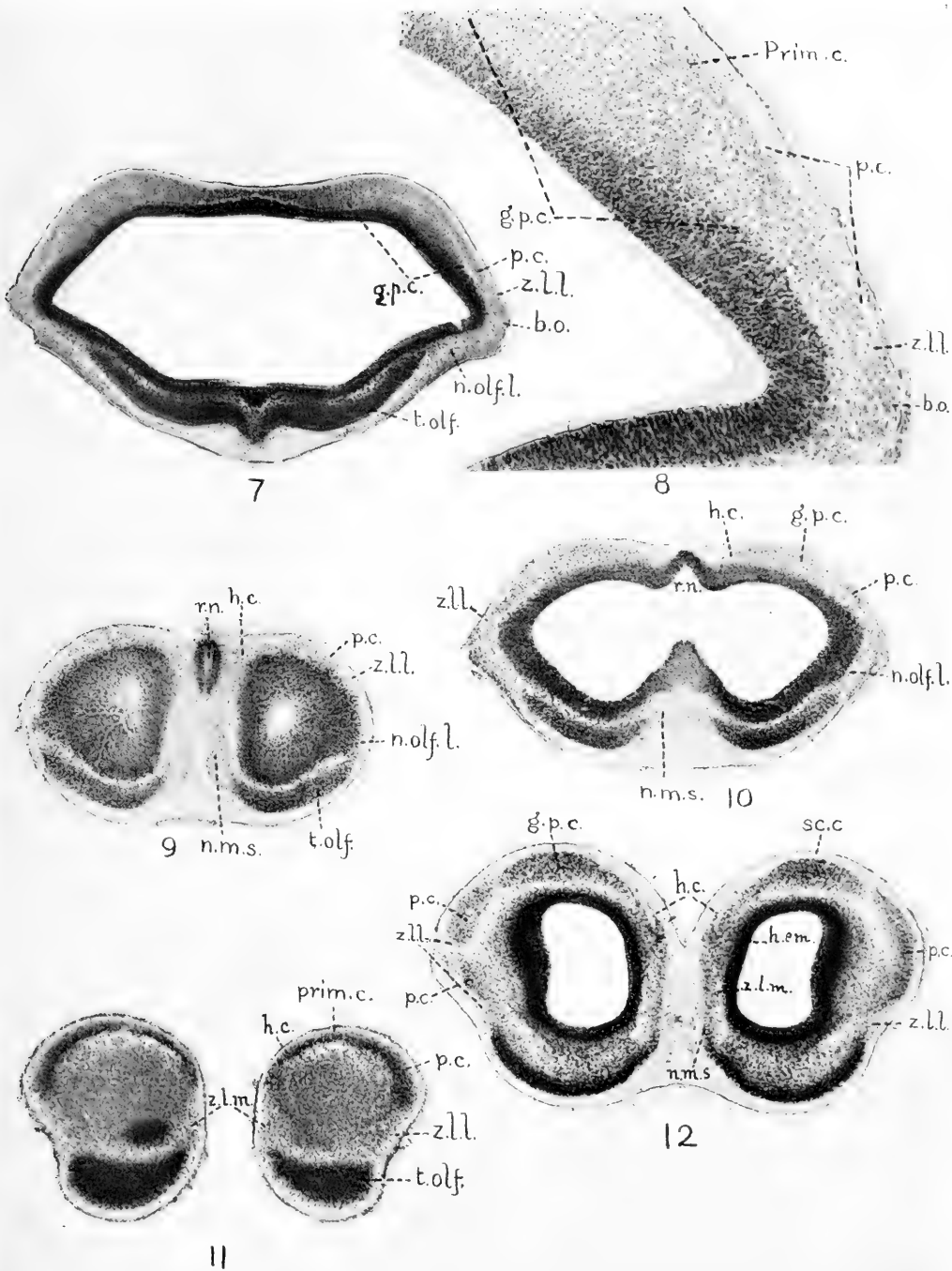


PLATE 3

EXPLANATION OF FIGURES

- 13 Acanthias, 5 cm. Section somewhat behind the section in figure 12.
- 14 Acanthias, 5 cm. Section just in front of the foramen monroi. Same series as figures 11 to 13.
- 15 Acanthias, 5 cm. Transverse section through the olfactory bulb. Same series as figure 14.
- 16 Acanthias, 5 cm. Transverse section through the posterior part of the pallium. Same series as figure 15.

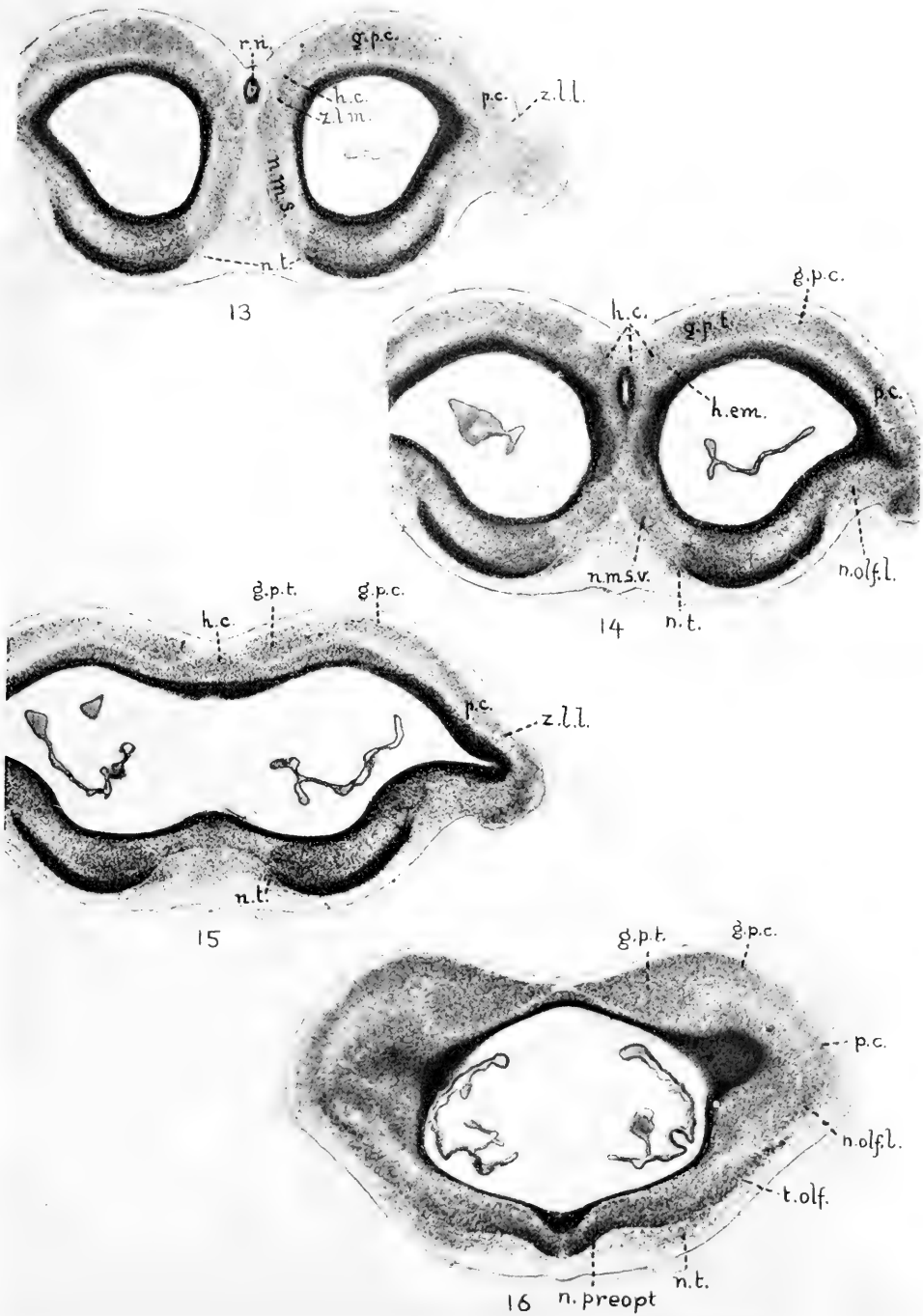


PLATE 4

EXPLANATION OF FIGURES

17, A, B Acanthias, 6.5 cm. Transverse sections taken, A, at the front pole of the hemispheres, B, somewhat before the foramen monroi.

18 Acanthias, 8 cm. Transverse section through the foremost part of the hemispheres.

19 Acanthias, 8 cm. Transverse section somewhat behind figure 18. Same series.

20 Acanthias, 8 cm. Transverse section somewhat behind figure 19. Same series.

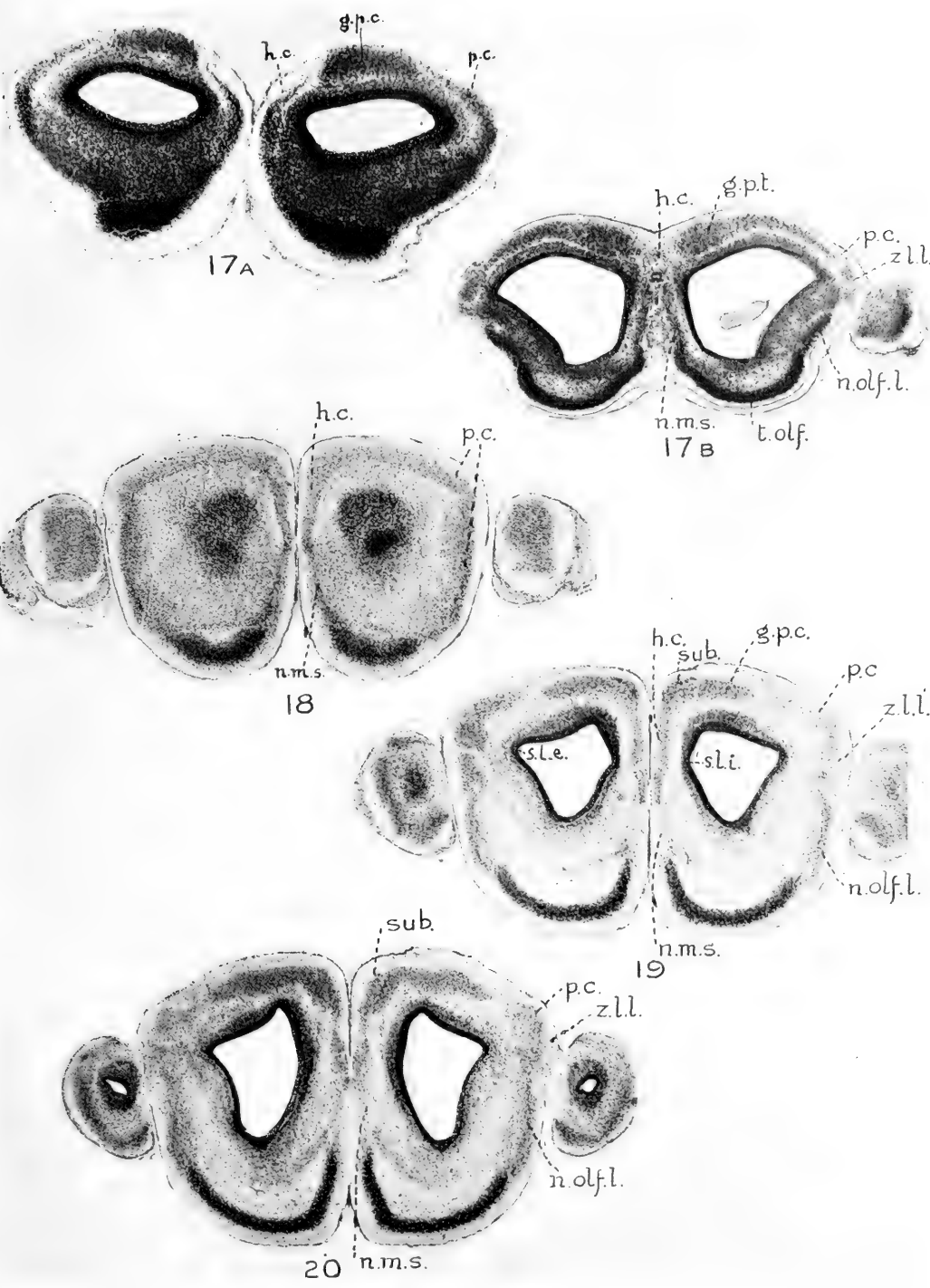


PLATE 5

EXPLANATION OF FIGURES

- 21 Acanthias, 8 cm. Transverse section somewhat behind figure 20. Same series.
- 22 Acanthias, 8 cm. Transverse section at the foramen monroi. Same series.
- 23 Acanthias, 8 cm. Transverse section through the posterior part of the pallium.

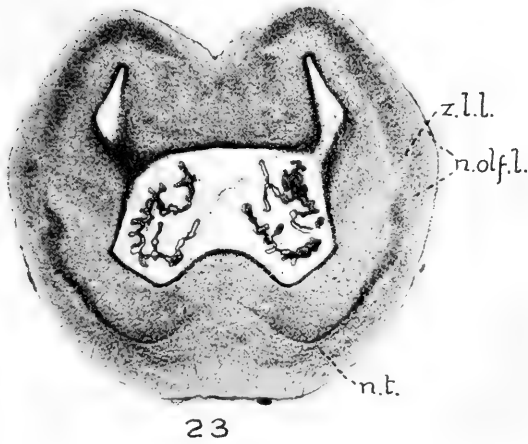
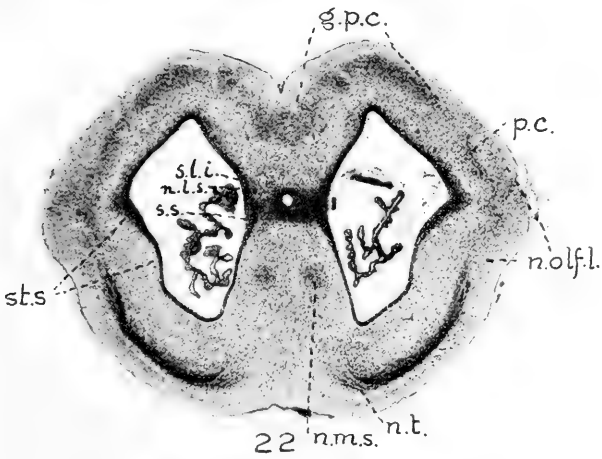
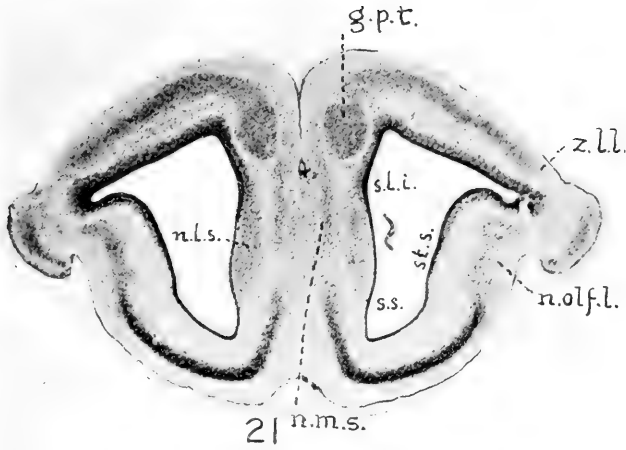


PLATE 6

EXPLANATION OF FIGURES

- 24 Chimaera. Transverse section through the evaginated part of the fore-brain.
- 25 Chimaera. Section taken somewhat behind figure 24. Same series.
- 26 Chimaera. Section taken just in front of the foramen monroi. Same series.
- 27 Chimaera. Section taken a little behind figure 26. Same series.
- 28 Chimaera. Section through the posterior portion of the pallium. Same series.

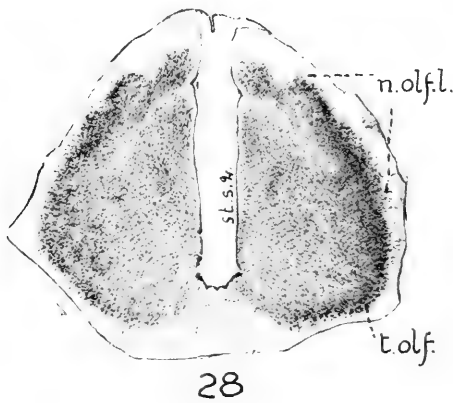
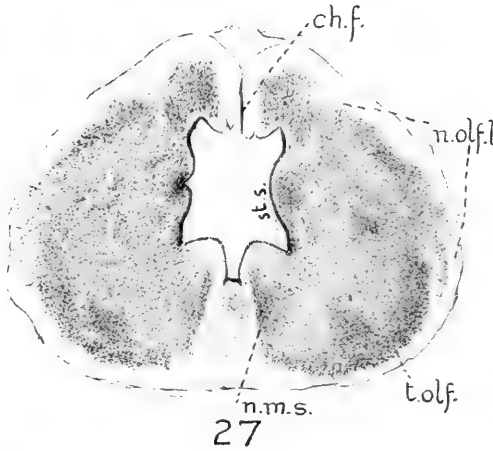
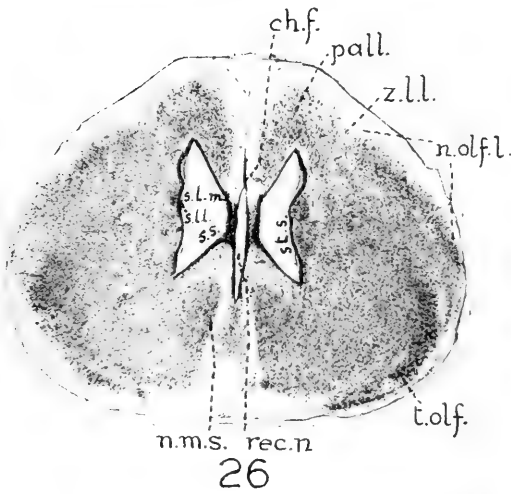
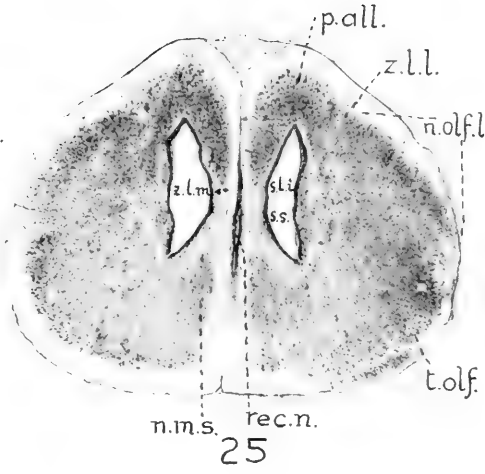
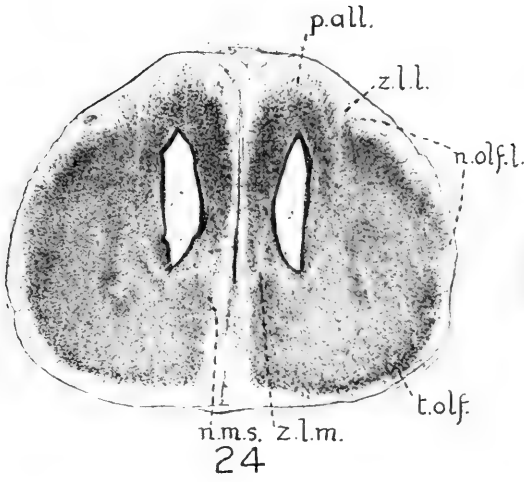


PLATE 7

EXPLANATION OF FIGURES

- 29 Protopterus. Transverse section taken through the rostral part of the pallium.
- 30 Protopterus. Section taken somewhat behind figure 29. Same series.
- 31 Protopterus. Section taken somewhat behind figure 30. Same series.
- 32 Protopterus. Section taken somewhat behind figure 31. Same series.
- 33 Protopterus. Section taken just in front of the foramen monroi. Same series.
- 34 Protopterus. Section taken through the foramen monroi. Same series.

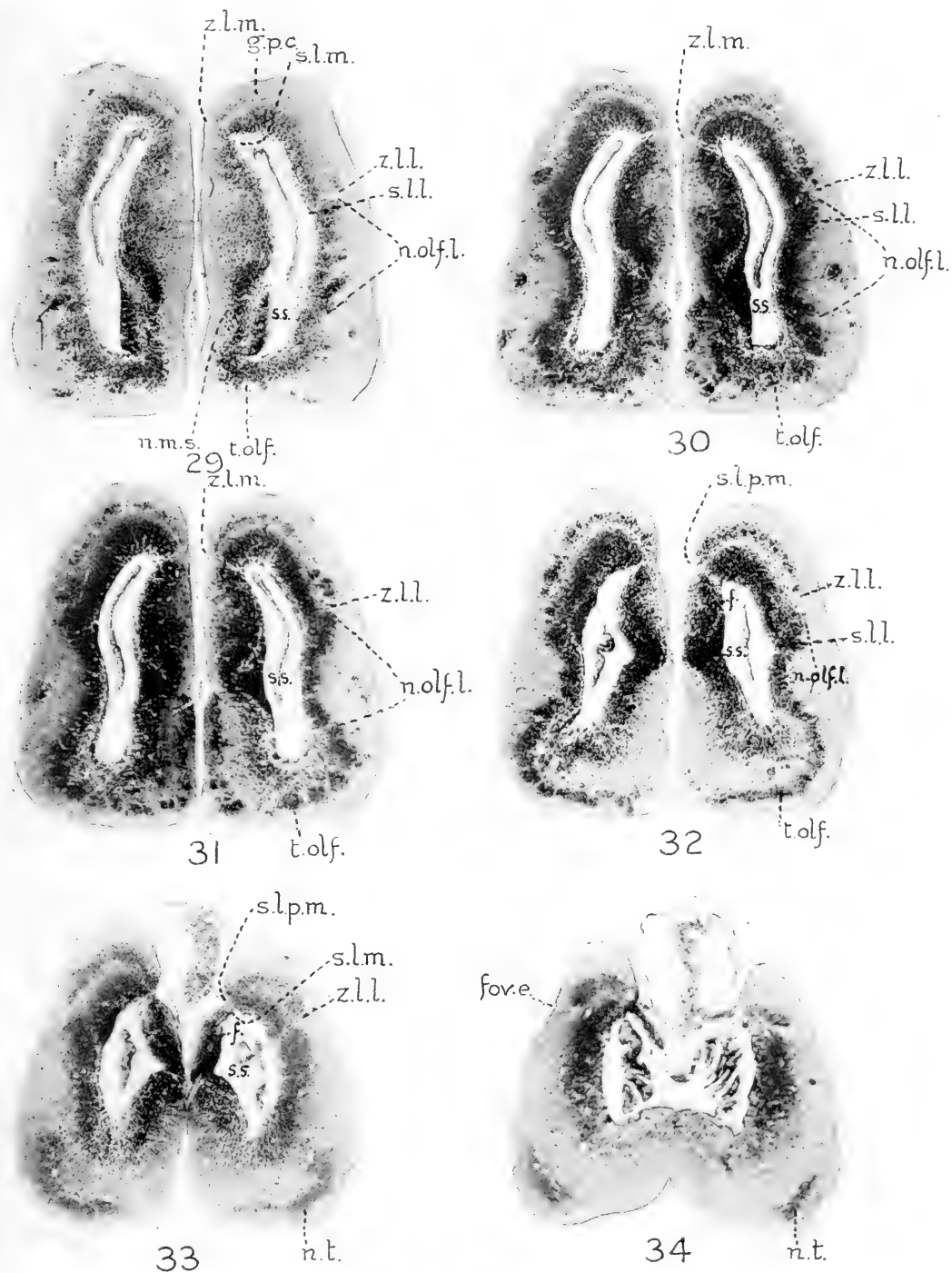


PLATE 8

EXPLANATION OF FIGURES

- 35 Polypterus. Transverse section through the olfactory bulb.
- 36 Polypterus. Section taken through the interventricular foramen. Same series as figure 35.
- 37 Polypterus. Section somewhat behind figure 36. Same series.
- 38 Polypterus. Section somewhat behind figure 37. Same series.
- 39 Lepidosteus. Section through the forebrain a good distance before the anterior commissure.

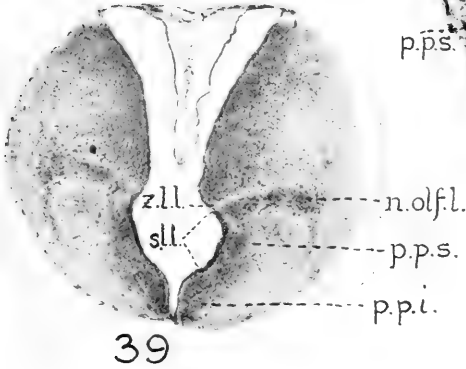
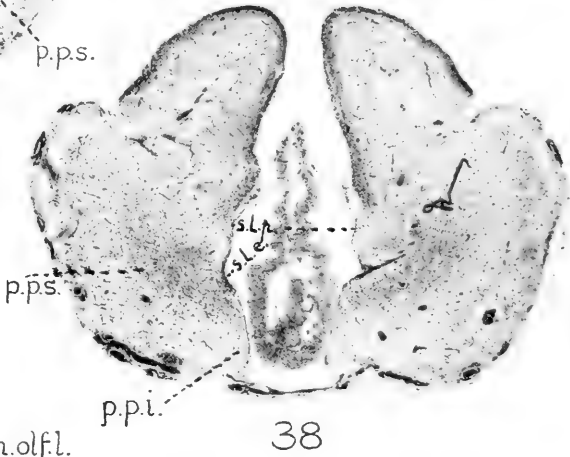
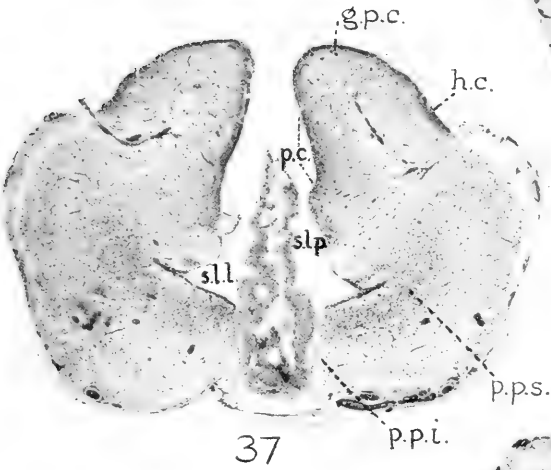
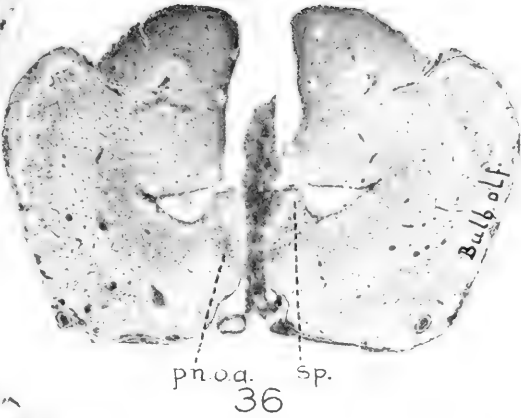
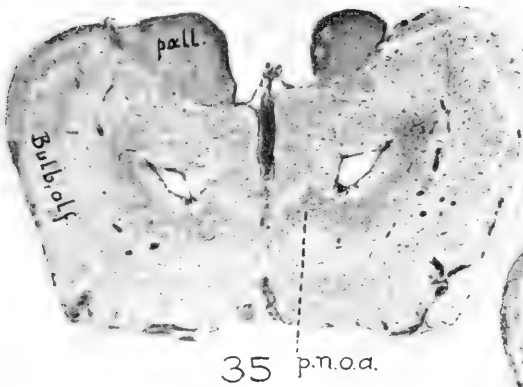
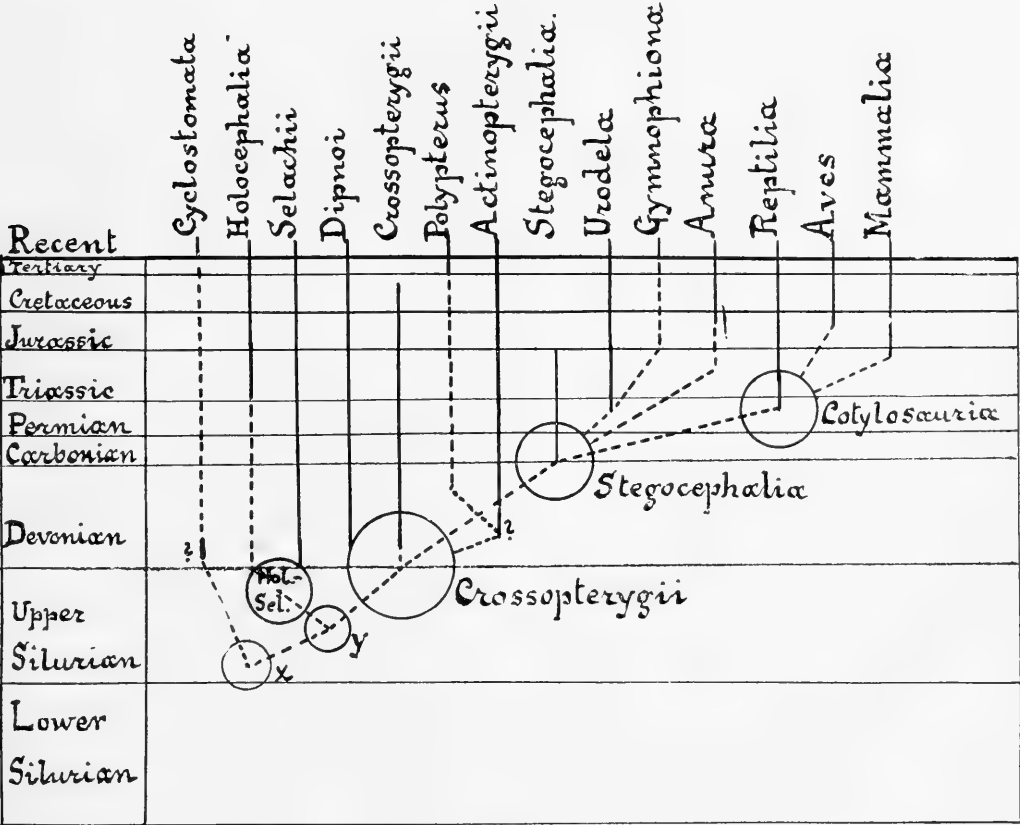
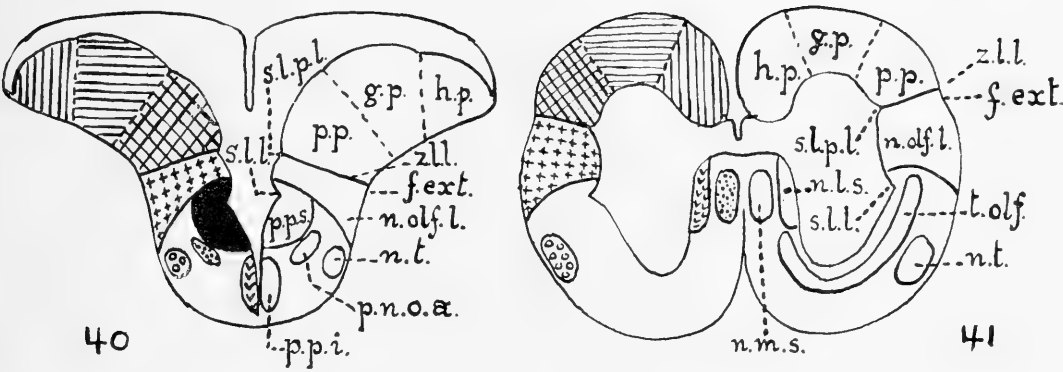


PLATE 9

EXPLANATION OF FIGURES

- 40 Scheme of the forebrain nuclei of the everted type.
- 41 Scheme of the forebrain nuclei of the inverted type.
- 42 Diagram of the vertebrate phylogeny.



Abstracted by B. F. Kingsbury, author
Cornell University, Ithaca.

The fundamental plan of the vertebrate brain.

This paper examines conceptions of the fundamental plan of the brain and brain-plate from the standpoint of the occurrence and significance of the three sutures postulated by His (sutura dorsalis, anterior (terminalis, frontalis), neurochordalis). Chick and shark (*Squalus acanthias*) were the forms chosen for examination. The study supports the analysis of the brain-plate presented in an earlier paper by the author (Jour. Comp. Neur., vol. 32, pp. 113-135), and confirms the interpretation of Johnston, a) that the brain-plate ends anteriorly (cephalically) with the terminal ridge embodying a potential chiasmatic ridge; b) that the two optic foveae (vesicles) are connected across the median plane by a primitive optic furrow which is also the primitive infundibulum (so-called). This relation but expresses the mechanics of growth. The approach was from the embryologic side, and it is indicated that the plan of the brain outlined and the early development of the brain are in full accord with the morphogenesis of the head as a whole.

THE FUNDAMENTAL PLAN OF THE VERTEBRATE BRAIN

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THIRTY-THREE FIGURES

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INTRODUCTORY

In a recent paper (Kingsbury, '20) I pointed out that many reasons both from fact and theory called for a fundamental modification of the primary morphological plan of the brain as given by His ('88, '89, '92, '93) and commonly presented by current text-books as established. The His conception of the brain was that of a primitively tubular structure (cf. His, '88, fig. 12). With the development by His of the very useful and fundamental idea of the existence of paired primary motor and sensory zones medially united by lines of suture, and the incorporation of this with the earlier interpretation of the brain as a tube, the logical conclusion was that these zones extended throughout the tube and that the ventral and dorsal sutures of the neural tube, marked by floor plate and roof plate, were at the anterior end of the tube supplemented by a third, terminal, suture (frontale Endnaht). In the discussion that follows these postulated or demonstrated lines of closure will be referred to as sutura neurochordalis (ventralis), sutura dorsalis, and sutura terminalis (anterior), respectively.

The His conception fitted in excellently with the already well-established view of brain segments;¹ since if the brain is composed of three 'primary segments' in turn divisible into five or six 'secondary segments,' these would only possess a segmental value if they were subdivisions of a fundamentally tubular structure and were serially comparable and metamerically equivalent. His therefore proposed a revision of the subdivisions of the brain—partly it is true for nomenclatural reasons—and his grouping and subdivision of the brain set forth in 1893 is the one generally accepted and the usual basis for text-book presentation of the brain. Both Herrick ('10) and Johnston ('09) have proposed modifications of the His schema of brain segments.

Likewise, the attempt to analyze the neural tube in terms of more primitive segments comparable with the somites of the mesoderm, the so-called 'neuromeres' found in the tubular plan of the brain an essential foundation for the interpretation, since clearly these could only possess value as primitive neural segments if they extended transversely to include the entire tube wall. Since to each neuromere there should belong primitively a motor and a sensory nerve the problem of the cranial nerves is also involved, as well as the 'primitive segmentation of the head.'

Since the publication of the papers by His dealing with the fundamental plan of the brain there have appeared three definite interpretations departing in important respects from his own. These are, in sequence, those of Johnston ('09), Schulte and Tilney ('15), and my own ('20). In order to bring out clearly the differences in interpretation and the points requiring verification two series of diagrams are presented: 1) of the brain in medial

¹ The history of the interpretation of the brain as a tubular organ composed of successive segments is practically coextensive with the knowledge of its development and comparative anatomy. While still earlier observers, apparently note the brain vesicles, to von Baer ('37) may be ascribed the recognition of three primary and five secondary brain vesicles with the well-known designations of the German vernacular—Vorderhirn, Zwischenhirn, Mittelhirn, etc. Huxley ('71) I believe introduced technical designations for these. Balfour, Wilder (B. G.), His, and von Kupffer each modified the scheme of segmentation in accordance with his own conceptions.

section, 2) of the brain plate (figs. 1 and 2). The latter series is essential in the comparison, inasmuch as it is the cephalic portion of the neural plate which by growth and folding produces the brain. It should, of course, be appreciated that these diagrams are purely schematic and intended merely to present the respective interpretations. The critical landmarks in the comparison of these interpretations are the mammillary recess (*M*), the primitive infundibular recess (*I*), the preoptic recess (optic recess) (*O*), and the extent of the floor plate (*F*). These points are designated in both series of diagrams by the significant letter. The floor plate is cross-lined, lamina basalis sparsely dotted, the lamina alaris is clear, while the line of secondary closure converting the brain plate into the vesicular brain is outlined by heavy dots.

The His interpretation (B of figs. 1 and 2) requires little explanatory comment. The floor plate (*F*) and his sutura neurochordalis terminated at the primitive infundibular recess (*I*) which marked the anterior medial limit of the brain plate. Anterior to this the sutura terminalis closed the neural tube in front, meeting the sutura dorsalis (roof plate). The line of demarcation (sulcus limitans) between the primary motor zone (*B*) and the primary sensory zone (*A*) terminates at the preoptic recess (*O*). His' own diagrams which are, of course, well known were copied in my earlier paper and may be compared.

Johnston ('09), entirely correctly as I think will be adequately shown later, placed the anterior limit of the brain plate at the preoptic recess.² C of figures 1 and 2 conveys his interpretation. Apparently he conceived the floor plate as extending throughout the brain plate, i.e., to the preoptic recess (cf. Kingsbury, '20, p. 122).

Schulte and Tilney ('15), from a study based on twenty-six models of the brain in embryo cats, came to conclusions unique in two respects: *a*) the brain plate (and the floor plate) were believed to extend no farther forward than the mammillary

² Mrs. S. P. Gage ('05) earlier came to the same conclusion (p. 426): "The natural corollary follows that the optic chiasma crosses the original margin or dorso-mesal line."

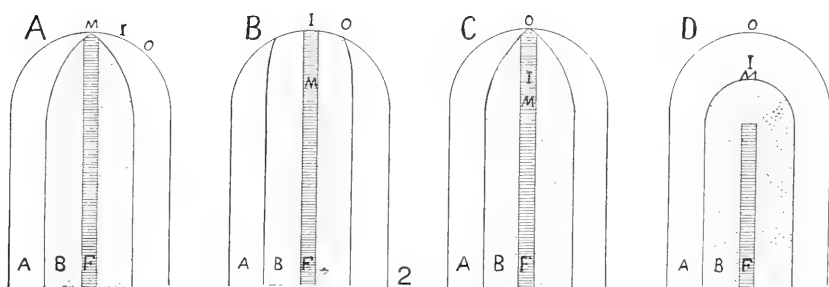
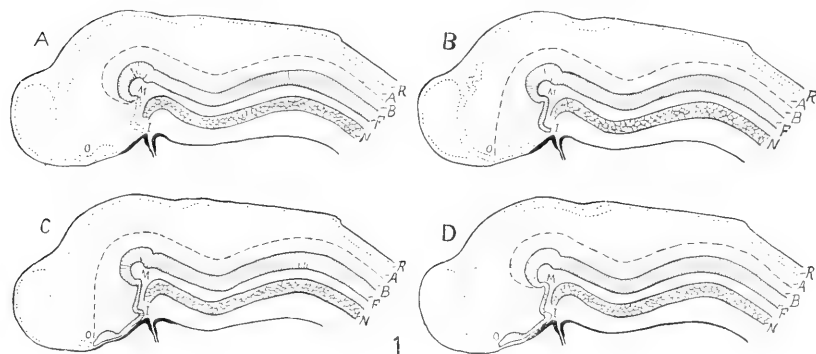
region (*M*). The line of secondary closure (of the neuropore, i.e., sutura terminalis) would be correspondingly increased in extent so that not only the optic chiasma, but the entire infundibular region would be thus included. A distinct advance was their recognition that the sulcus limitans could not be traced forward beyond the mammillary recess. *b*) The second unique feature of their interpretation was the inclusion within the limits of the more cephalic portion of the alar plate of neural crest elements. As this does not closely concern the aspects considered in this paper, no discussion will be given, nor is there in A of figures 1 and 2, which present their views, any diagrammatic expression of this side of their interpretation.

My own interpretation is represented by D, figures 1 and 2. There it may be seen that the floor plate extends no farther forward than the fovea isthmi (cf. Kingsbury, '20), whereas the medial cephalic limit of the brain plate would include the optic chiasma. The unique feature of the interpretation and the one on which in my opinion its value largely rests is that of a primary continuity across the middle plane of 'nervous material'—alar and basilar plates—anterior to the fovea isthmi and floor plate, to both of which structures considerable morphological significance is believed to attach. In figure 1, D, the 'prefoveal' extent of the brain plate is indicated by heavy stippling.

Thus each of these interpretations, although differing quite fundamentally, made I think a distinct advance toward an understanding of the correct relations. My earlier paper outlines certain of the bearings of the interpretation offered by me. These it will be seen affect not alone or mainly the brain, but possess a broader bearing. The approach to the interpretation was from the embryological side rather than the neurological, and the writer's interest has centered not so much in affording a satisfactory basis for analyzing the brain as determining the developmental pattern³ of the body. The early morphogenesis

³ For a number of years (cf. Kingsbury, '13) I have recognized the importance of distinguishing Process and Pattern as two fundamental aspects of development as of life generally. Recently Child ('21) has likewise recognized the validity of the distinction, this time drawn between Pattern and Material, however.

of the brain cannot adequately be considered by itself. The problem of the development of the brain is also that of the head and the primitive plan of the brain plate, and the growth changes that transform it into the vesicular brain must be in full harmony with the facts of development for other cranial structures.



Figs. 1 and 2 Schemata to illustrate the interpretations of Schulte and Tilney (A), His (B), Johnston (C), and Kingsbury (D), of the fundamental plan of the brain and brain plate, respectively, in terms of primary motor and sensory zones. A, alar plate; B, basal plate; F, floor plate; I, primitive infundibulum; M, mamillary recess; N, notochord; O, preoptic recess; R, roof plate.

Furthermore, neither the brain nor the head can be considered by itself developmentally or for that matter phylogenetically and any adequate interpretation of the developmental plan of the head and brain must be in full harmony with the developmental pattern of the body as a whole. Also I venture to add it should be appreciated that in as much as all vertebrates conform in the fundamental structural plan of the adult body, there must likewise be conformity in the developmental plan. In other words, the differential growth of all vertebrates must of very necessity follow the same pattern. The full significance of this is I think frequently overlooked. It is quite essential that the developmental pattern be established as well as the developmental processes ascertained.

Without considering structural aspects purely neurological wherein the interpretation 'D' would clarify difficulties, there are certain general questions of importance in the choice of interpretations that may be answered by an appeal to the facts and which the present paper aims to consider. These are: *a*) The determination of the cephalic end of the primitive brain plate. Johnston, followed by Kingsbury it will be recalled, included the chiasma; His terminated it at the infundibular recess, Schulte and Tilney at the mammillary recess. The existence of the primitive optic furrow of Johnston. *b*) The question as to the existence and extent of a sutura terminalis as distinct from a sutura dorsalis. *c*) The significance of the sutura neurochordalis of His and the primitive extent of the floor plate. In the examination of these specific questions the general significance of conception 'D' of figures 1 and 2 will come out in clearer outline.

Material. In the choice of forms in which to test the questions, two were selected for consideration in this paper—the shark (*Squalus acanthias*) and the chick. Important considerations here were: *a*) the availability, since it is quite important that a close series of stages at critical epochs be at hand; *b*) ease of orientation. Medial sagittal sections through the head are quite essential. Model reconstructions from transverse series alone are far less useful and trustworthy. A considerable number of

series cut in the sagittal plane (for the head) at important stages were prepared supplementing the series already available in the collection of the Department of Histology and Embryology, Cornell University, and those of Prof. S. H. Gage which he kindly placed at my disposal. In addition, series were prepared for and by Mr. H. B. Adelmann for his investigation of the origin of head mesoderm. His studies have materially aided the present study and his paper will be referred to subsequently and due acknowledgment made. c) Both of these forms have been previously utilized in important studies of the early development of the brain and, while the literature in itself afforded no complete basis for conclusions, its existence was nevertheless helpful. d) The two forms belong to quite remote classes of vertebrates.

OBSERVATIONS

1. The extent of the brain plate and the primitive optic furrow

Squalus acanthias. In addition to the qualifications above mentioned, the shark possesses the important feature of a sharply defined brain plate. The early development of the brain of the shark has been investigated by a number, particularly von Kupffer ('06), Loey ('95), Neal ('98), and Johnston ('09). Despite this fact, there was not furnished in the literature sufficiently exact information to permit without repeated examination answers to the questions proposed.

To determine the first point at issue, i.e., the extent of the brain plate, the location of the retinal areas and the existence of a primitive optic furrow (Johnston), surface views of a close series of shark blastoderms were examined, photographed at a magnification of ten diameters, and sectioned, usually in the sagittal plane, and a careful medial plane reconstruction prepared. Certain of these surface views are reproduced as figures 6 to 16. Middle plane reconstructions from the embryos of figures 6, 11, and 13 are given on plate 2 as figures 17, 19 and 20, respectively, while plottings of neural plate, notochord, mesoderm and entoderm from embryos of figures 7 and 9 are given as text-figures 3 and 4.

Johnston ('09), it may be recalled, gave evidence that, *a*) the retinal foveae (evaginations) were connected across the median plane by a furrow, the same which His might have termed the 'basilar furrow' as the internal counterpart of his 'Basilarleiste.' This furrow Johnston concluded might persist as a postoptic recess, while the infundibulum was a secondary development. The optic chiasma he found occupied the cephalic border of the brain plate (cf. Kingsbury, '20, p. 122).

A merely cursory inspection of figures 9 to 13 of plate 1 and the corresponding median plane reconstructions of plate 2 (figs. 18 to 22) suffices to confirm the conclusions of Johnston on the first point, since it is evident that his first transverse furrow on the brain plate is continuous with the retinal foveae and later with the optic vesicles, and therefore well deserves the name of 'primitive optic furrow.' It would seem to me, however, that it also constitutes the primitive infundibulum⁴ and is in fact at one and the same time primitive optic furrow and primitive infundibular furrow. While it is probable that in the unequal growth of the region it becomes, as Johnston affirms, the relatively insignificant postoptic recess, I have not noted any indications that such is the case, doubtless because sufficiently late stages have not been examined. Although it differentiates relatively late, the same comparison of figures indicated quite clearly that the chiasma is included within the extent of the brain plate. In the later stages medial sections (figs. 23 to 26) alone are not conclusive, but, interpreted from the relations of the optic stalk in the more lateral sections, they sustain Johnston's conclusions in this regard also.

⁴ Johnston's contention that the definitive infundibulum (i.e., depression leading to and into the cavity of the pars nervosa of the hypophysis) is a secondary or later development is of course entirely correct. Embryologists have, however, so generally applied to this primitive recess the term infundibulum that perhaps for a time at least the designation may be retained, but distinguished as the 'primitive infundibulum' or 'primitive infundibular recess.' It should be appreciated that it is but an expression of the mechanics of growth of brain and head in their early morphogenesis. The term 'hypothalamus' seems to the writer to apply more exactly to the topography of the wall and thus leave a need for an additional and supplementary term. The question of the nomenclature of this portion of the diencephalic floor is discussed by Tilney ('15).

On the other hand, no confirmation is afforded for the views of Schulte and Tilney. According to these authors, in the cat there exists in the neural tube a landmark of importance—the ‘tubercle of the floor.’ This marks the anterior end of the neural plate, thus forming the ventral lip of the neuropore and also the anterior end of the floor plate. Ventrally the tubercle of the floor is “in relation with the blind extremity of the foregut (while) the stomodaeum approaches but hardly reaches it in front” (p. 339). In later stages “it forms a transverse ridge intervening between the mammillary and infundibular regions.” The optic vesicles at an early stage form the cephalic extremity of the neuraxis and between these there exists an ‘optic sulcus’ crossing the mid-plane anterior to the tubercle and partially interrupted (caudally) by it. The optic vesicles during growth, as they interpret, decrease in size contributing the infundibular region caudally and the anlagen of the thalamencephalon and telencephalon anteriorly (cephalically). The line of closure of the neuropore they thus consider very extensive, including lamina terminalis, optic chiasma, and infundibulum as well.

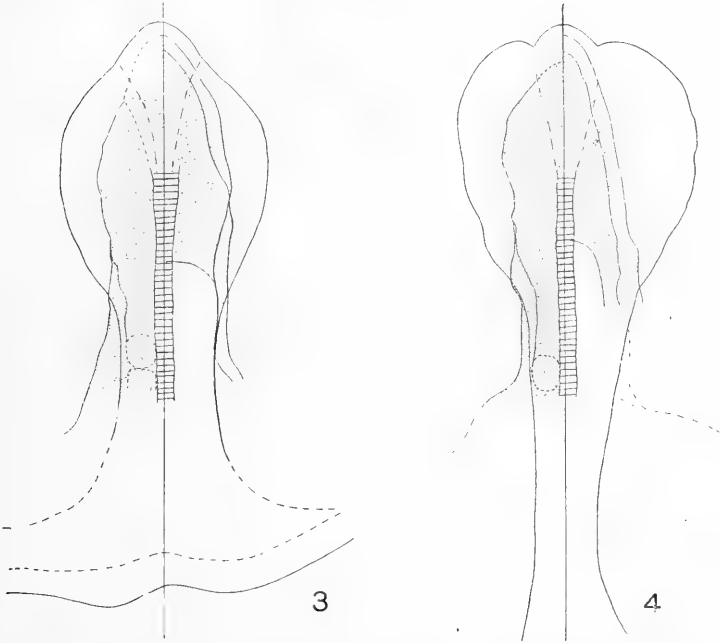
As stated in my earlier paper, the critical point is the interpretation of the ‘tubercle of the floor,’ and it was there commented that the figures offered by them in illustration “neither conclusively show that it corresponds to the anterior end of the neural plate nor that it marks the anterior boundary of the mammillary recess.” As to the first point above it may be urged that their figure 1 of plate 27 (cat of four somites in which the neural folds have not yet fused) shows, on the contrary, that there intervenes between the ‘tubercle of the floor’ and the anterior end of the brain plate the primitive optic furrow (their ‘optic sulcus’) and a terminal ridge in which, in the light of Johnston’s work and my own observations, I should be inclined to see a chiasmatic ridge. Their figure (1 of plate 27) might well be compared with figure 10 (9 and 11) in this paper of the neural plate of the shark. Flatten out the neural plate as it is shown in their model in the cat and the equivalence would be quite striking.

The shark, however, possesses no well-defined ‘tubercle of the floor.’ The slight elevation, which from their description of the

relations the tubercle of the floor bears to foregut and stomodaeum would correspond to it, is marked in figure 20 by the letter *T*. It also agrees with the tubercle of the floor in being caudal to the primitive infundibular recess. This slight elevation in the shark is clearly but the mechanical expression of the underlying 'preaxial mesoderm.' In the neural plate stages, figures 7, 8, and 9, there is indicated, somewhat faintly it is true, a wedge-shaped area⁵ tapering caudally from a rounded 'apical lobe' which from its photographic reproduction might easily be interpreted as a typical 'tubercle of the floor' extending quite to the anterior edge of the neural plate. The comparison of the Schulte and Tilney figure 1, plate 27, with figure 10 of this paper, already called for in connection with the optic sulcus, might be again appealed to as establishing the equivalence of this wedge-shaped area with the tubercle of Schulte and Tilney. However, examination of transections shows that in such stages as those of figures 9, 10, and 11 there is no or but slight elevation of the neural plate and no marked thickening of the neural plate which corresponds to the area reproduced by the photograph. Plottings were made of mesoderm, entoderm, notochord, and neural plate showing their position and extent in the embryos of figures 7 and 9, and these are reproduced here as figures 3 and 4, respectively. From their examination it is quite evident that the appearance seen in the surface examination of shark neural plates and which the camera reproduces is mainly an expression of the greater thickness of the underlying preaxial tissues where entoderm, mesoderm and notochord are indistinguishably united—a region with whose significance Adelmann ('22) has more particularly to do. The progressive transformation of this region as seen in the median plane from these or comparable stages may be seen in the comparison of figures 17 to 21 (plate 2).

⁵ This anterior medial wedge-shaped 'area' was noted and figured by both Loey and Neal. The former says regarding it (p. 551): "This has already been spoken of in Part I as a tongue-like process extending from the median anterior tip backwards to two-thirds the length of the cephalic plate. It continues to be a prominent feature of the cephalic plate for some time. I can offer no suggestion as to its significance, outside the obvious suspicion that it may represent a proboscis of some kind, or that it may be related to the large notochord of this region."

The 'apical lobe' is intimately related to the 'pre-oral' extension of the foregut, and it is the great expansion of the more lateral region of the neural plate in this region that sets it off as an apical lobe.



Figs. 3 and 4 Plottings from series cut in the transverse plane of *Squalus acanthias* embryos, 2.2 mm. and 2.6 mm. length, 6 and 8 somites, respectively. These are the embryos of figures 7 and 9, plate 1. In each, the notochord is indicated by cross-barring; the stipple areas on the right and left mark the extent of the entoderm and mesoderm, respectively; the two lines on the right bound the lateral wall of the archenteric cavity; the outline of the median 'wedge-shaped area' is shown in broken line, and closely corresponds to the territory of the prechordal plate at these stages.

Whether the tubercle of the floor in the cat is a mechanical expression of a moulding of the neural plate upon the "blind extension of the foregut" cannot be determined from the figures of Schulte and Tilney, but it seems hardly probable. A mechanical crowding in the forward growth seems more likely to be responsible for its presence.

Chick. While in the chick embryo the brain plate is less clearly defined in the early blastoderm and the retinal foveae do not appear until after the neural folds have formed, fundamental agreement with the relations existing in the shark nevertheless exists.

Despite the large amount of work done on the development of the chick, adequate descriptions of the early relations and transformations of the neural plate are lacking, and it was necessary to undertake a detailed study of a sequence of stages. Eighteen median plane reconstructions from sagittal sections were made by Mr. Adelman and myself, of which six are here shown in accurate outline, but diagrammatically reproduced, as figures 12 to 32. Of these, the reconstructions of figures 27, 29, 30, and 32 were also utilized by Mr. Adelman in his paper, who added four additional ones.

Johnston ('09) does not describe the condition in the chick in detail, but it has been found that in this form as well as in the shark the primitive infundibular furrow is also a primitive optic furrow. The earliest stage in which the furrow appears is one of six to seven somites (fig. 27). Here we see two slight furrows with equally slight prominences overlying a region where notochord mesoderm and entoderm are blended and intimately in contact (at least) with the neural plate. An earlier stage (four somites) illustrates the above-mentioned important characteristics of this important region without the presence of these furrows or slight depressions. There is an obvious pushing forward and downward of this region in the growth expansion. The more anterior of these furrows becomes the primitive infundibular furrow, as may be seen by comparing figures 27, 28, 29, and 30. In the interval of development which they represent the optic vesicles have appeared, and by tracing the infundibular furrow to either side it is evident that it leads directly into those lateral expansions of the tube, fully substantiating Johnston's interpretation. The community of relation of optic vesicle (furrow) and primitive infundibulum which exists in the interior of the tube has an external counterpart, as is well shown in the His models of the developing chick brain where optic vesicle and

infundibular fold are continuous with one another. As the brain expands the primitive infundibulum becomes extended cephalo-caudally and loses its direct and primary communication with the optic vesicles (figs. 30, 31, 32).

A comparison of these figures (figs. 27 to 30) will also substantiate the important point that the primitive infundibulum (primitive optic furrow) is within the territory of the brain plate. The preoptic recess appears later (fig. 32). From the relations, which were well ascertained by Johnston ('09), it is apparent that the optic chiasma, although it appears only subsequently, marks the anterior boundary of the optic furrow and the anterior limit of the brain plate medially.

From the examination of the relations in the chick, as in the case of the shark, the interpretations of Schulte and Tilney lack substantiation. In the chick, as in the mammal (e.g., cat, Schulte and Tilney), the early formation of the neural folds disguise relations that are clearer in the shark due to the greater expansion of the brain plate (cf. figs. 9, 10, 11) before the neural folds rise up. Were one to 'open out' the neural folds in, say, the four- to six-somite chick as in the four-somite cat of Schulte and Tilney, the morphological identity would be quite striking.

The prominences seen in the median plane reconstructions of the chick (figs. 27 to 30) correspond closely to Schulte and Tilney's tubercle of the floor. Whether the second (slight) furrow corresponds with the future mammillary recess was not determined. I am inclined to regard these prominences—as indeed the furrows—as but expressions of the growth mechanics of the region and caused mainly by a crowding forward.

2. *The sutura terminalis (anterior)*

In the His conception of the brain as a tube a separate anterior closing sutura, sutura frontalis (terminalis, anterior), 'frontale Endnaht,' would have a distinct morphologic significance. If, however, it is recognized that the brain is developed from a plate folded together while it grows, such a theoretical significance would not attach to this portion of the secondary line of closure. As is well known, the dorsal suture is first formed somewhere

in the neighborhood of the tectum mesencephali, although there is clearly considerable variation in this respect. The closure of the neural tube progressing cephalad (as well as caudad) leaves a slit-like opening of the neural tube anteriorly (chick of eight to nine somites; cf. figs. 27 to 29). Its ventral extremity lies at the site of the future preoptic recess. Due to the expansion of the dorsal portion of the neural tube in this region, it is from the beginning in part ventral in position. Figure 33 is from a model of the anterior portion of the head in a chick of eight to nine somites as seen from the ventral side. Its ventral extremity likewise marks the anterior medial boundary of the neural plate. The suture closes rapidly and apparently last at or near its dorsal extremity, although again there appears to be some variation in different forms and individually. To this entire opening from the earliest stage the term 'neuropore' is frequently applied, while others would limit the term to the point of final closure. Possibly it would be well to distinguish these as primitive neuropore and definitive neuropore, respectively.

Inasmuch as the bordering edge of the neural plate in this region marks the lamina terminalis, it would seem that a separate designation would be of value for descriptive purposes. It should, however, be appreciated that essentially it is but the most anterior portion of the sutura dorsalis given a peculiar significance through the growth expansion of the adjacent portion of the brain (and head).

It may be remarked that Lillie ('19) correctly describes in the chick the anterior relations of the sutura terminalis (pp. 105, 147). The interpretations of Schulte and Tilney ('15) are as obviously wrong. Johnston ('11) has quite adequately discussed this region (p. 39) and with what he there states my observations agree.

3. *The sutura neurochordalis (ventralis)*

The His conception of the origin of the body through the apposition or concrescence of a germ ring is of course well known. The idea was an old one with him and appears as early as 1874, prompted by his work on the development of the salmon and

strengthened by examination of the shark ('77). Apparently the entire vertebrate body was thought of as formed by concrescence in the midplane of right and left moieties, although by both word and figure he seems to have recognized a primitive continuity of material at the anterior end of the body axis. Inasmuch as the primitive line of junction established the neural plate externally and the chordal plate internally, His designated it as the 'neurochordale Naht.' This two-fold effect of concrescence His recognized as early as 1874 and comments on in 1877, but the designation was not given until he turned his attention to the structural plan of the central nervous system. Goronowitsch ('93) was apparently the first to give it technical form as the 'sutura neurochordalis (ventralis),' although he failed to grasp the significance of the term.

Hertwig ('92) gave a new significance to the neurochordal suture through his recognition that it marked the line of closure of the blastopore.

The interpretations of His and of Hertwig and their bearing have been, I think, adequately discussed in my earlier paper (Kingsbury, '20). It remains to examine more closely than was then attempted the value and significance of the term 'neurochordal suture' as applied to the brain.

The neurochordal suture, according to His, becomes in the neural tube marked out by the floor plate and this—in accordance with his conception of the origin of the body—extended to the anterior end of the neural plate. The distinction of neurochordal suture and floor plate should of course be appreciated. The neurochordal suture is a structurally negative term; an ideal plane of junction. The floor plate is a structural differentiation. The term may be used either in a purely descriptive way or as a structure of ontogenetic significance. Reference to my earlier paper (p. 115) is indicated in this connection.

The problem of the neurochordal suture may perhaps be stated in question form as follows: Does the floor plate mark in the neural plate (tube) the line of closure, either actual or potential, of the blastopore? Does the notochordal plate likewise coincide with the line of closure of the blastopore? If so, these two struc-

tures should be when first laid down coextensive. Since I have shown that a differentiated floor plate extends no farther forward than the fovea isthmi, the primary extent of the notochord would thus fall far short of the point marked out as its anterior end by His, namely, the point where sutura terminalis, sutura neurochordalis, ectoderm, and entoderm (i.e., pharyngeal membrane) adjoin (cf. the His fig. 1, 1892, 2 paper, reproduced in my earlier paper as figure 4).

The question as to whether or not it is permissible to recognize a primitive suture, actual or potential, uniting the right and left halves in the epichordal portion of the body is a purely embryological one and it is not proposed to enter into its discussion here. More pertinent at this time is the determination as to whether or not floor plate and notochordal plate are primarily coextensive, and hence whether, granting the propriety of the term suture, it may likewise be designated as neurochordal suture. This determination is somewhat difficult for two main reasons: 1) At the early developmental stage in which notochord and neural plate are in juxtaposition the floor plate is still undifferentiated. Likewise in most forms at least the anterior end of the notochord is not at first clearly demarcated from the prechordal plate (preaxial mesoderm). 2) The neural plate (tube) and notochord grow markedly and at different rates, the former obviously more rapidly, so that the neural tube becomes bent away and separated from the notochord so that by the time the fovea isthmi is evident and the floor plate distinguishable through its differential change, the points for comparison are quite remote from each other. These difficulties do not, however, render valueless a comparison of neural plate and notochordal plate and recourse may again be had to the median plane relations in progressively older stages.

Shark. Sixteen median plane reconstructions were made of shark embryos from 1.5 to 23 mm. length, of which ten are reproduced as plate 2. The magnification is the same for all so as to permit more readily visualization of the growth changes. Figures 17, 19, and 20 are from the embryos shown in figures 6, 11, and 13, respectively. Figures 18, 21, and 22 are of stages

intermediate to those of 9 and 10, 13 and 14, and 15 and 16, respectively. Figures 23 to 26 are of progressively older stages, all of them, however, older than that represented by figure 16.

While in figure 17 the notochord is included in the entoderm and its prospective cephalic end is indeterminate without a comparison with the mesoderm and with later stages, in the succeeding stages figured it is separated off save at its cephalic and caudal ends where prechordal plate and marginal zone (blastoporic lip) are united to it. Both of these zones mark regions where notochord, paraxial mesoderm and entoderm are confluent. In the case of the marginal zone, of course, the neural plate must be added to these. The prechordal plate is a region of great significance in understanding the morphology of the head. Without stopping to consider the neural plate-notochordal plate relations more closely at this point, it is obvious at once from a survey of these figures that not only does the anterior end of the notochord fail to reach the anterior end of the neural plate, but falls far short of the primitive infundibular fold, being separated from it by the greater extent of the prechordal plate (cf. figs. 17 to 20) in which it ends.

Turning to a more exact comparison of notochord and neural plate, in order to determine if possible the point in the neural plate with which the anterior end of the notochord primitively corresponds, it should be noted that only in the last figure of the series (fig. 26) is the fovea isthmi and the cephalic end of a differentiated floor plate distinguishable, where it has been designated by the letter *F*. The interval of the medial stretch of floor between this point and the tuberculum posterius of von Kupffer is in the shark embryo of this stage extensive. *P* marks the latter landmark. No significant alterations in form development take place between this stage and the 40-mm. embryo figured in my earlier paper (figs. 1 and 8), with which it may be compared. Each preceding earlier stage has been compared and the equivalent point similarly designated, back to and including that of figure 19. In this comparison the descriptions and figures of Neal ('98), von Kupffer ('06), and Scammon ('11) have been considered and compared, particularly the fine plates

of the former. The medial plane sections in each of these series have been compared with the parasagittal sections and the general plane of the external 'fissura rhombomesencephalica' indicated by lines. It appears clear that the expansion and pushing forward of the neural plate carries the point in question forward (and downward) (figs. 18, 19, 20). Subsequently the (cephalo-caudal) expansion of the primitive infundibulum and the increase in extent of the mesencephalic floor shifts it relatively backward (figs. 20, 21, 22, 23) to again advance it with the growth of the rhombencephalon (figs. 24, 25, 26). In comparing figures 20 to 24, the relations of the second head somite material may be noted. In figure 20 the two short lines mark its cephalo-caudal extent as it lies paraxially. In figure 21 the neural plate has begun to separate from closer contact with the notochord in this region. In this space a dense mesenchymal growth from the second head somite has appeared in figures 22 and 23. From this somite much at least of the mesenchyme occupying the plica encephali ventralis is derived.

This comparison of a close series of stages indicates that the point of the prospective fovea isthmi in the neural plate corresponds closely at least to the anterior end of the notochordal plate. It does not seem possible more closely to establish the correlation, due to the differences in differentiation and growth earlier mentioned, without resort to an extensive and supplementary series of models of the region, or experimentally. The point could hardly be tested experimentally in shark or chick. In the Amphibia, however, this would seem quite possible, where, it might be incidentally remarked, I have found (in *Amblystoma punctatum*) conformity to the developmental pattern of the head outlined here for shark and chick. The comparison instituted entirely suffices, however, to establish clearly the incorrectness of the His diagram (His, '92, fig. 1; cf. Kingsbury, '20, fig. 4), in which the notochord extends to the infundibular fold and to the anterior end of the neural plate. The His conception of the brain plate, therefore, which the diagram illustrates fails of substantiation. The His term, 'neurochordal suture,' however, deserves, I think, retention, but

with full recognition that its use implies the acceptance of a line of blastoporal closure, actual or potential, with which it coincides, and which in the brain and spinal cord is marked by the floor plate whose anterior limit I have placed at the fovea isthmi.

Inasmuch as Johnston ('09) and Schulte and Tilney ('15) did not consider the growth of the brain plate in its relation to the notochord and head mesoderm, the question of the neurochordal suture and its extent is not directly involved in the adjudication of their interpretations. Doubtless in prolonging a floor plate forward to the anterior end of the neural plate they but expressed an acceptance of the His analysis of the neural tube.

It may be pointed out that the primitive furrow of the neural plate which appears in figures 6 to 11 and whose cephalic end is carried forward and down (figs. 11 and 12) and is lost to view with the development of the neural folds corresponds closely to the extent of the notochord. Figures 3 and 4, which are plottings to show the extent and relations of notochord mesoderm and entoderm in the embryos of figures 7 and 9, respectively, may be compared. This furrow I believe unquestionably corresponds to the neurochordal suture.

Chick. While there exists in the chick as in the shark the same fundamental plan of relations of neural plate, notochord, prechordal plate and entoderm, with the characteristic morphological transformations of forward growth and down-bending, it has not been possible clearly to determine the point of the fovea isthmi in the neural plate. It is obvious, however (figs. 27 to 32), that the notochord ends far from the anterior end of the neural plate separated from the infundibulum, as in the shark, by the extent of the prechordal plate, and at a level such as would, from the subsequent growth transformations in the brain, correspond closely with the level of the prospective fovea isthmi and the anterior end of the floor plate. The fovea isthmi and floor plate could not be detected, however, earlier than five days' incubation, and no serious attempt was made to bridge the interval by a detailed study of intervening stages. The essential similarity in the pattern in chick and shark may be seen by a comparison

of the figures of plates 2 and 3. The important relations of notochord and prechordal plate are more adequately discussed by Adelmann ('22).

CONCLUSIONS

In the foregoing pages the four interpretations outlined in the introductory paragraphs of this paper have been examined from the three points of view proposed on the question of fact, and the conclusion is safe, I think, that the His interpretation and that of Schulte and Tilney fail to satisfy the requirements. Johnston's observations have been confirmed in the essential feature that the neural plate terminates with the chiasmatic ridge and that the primitive infundibular furrow is likewise a primitive optic furrow. My own interpretation has added what I regard as an important point—the recognition of a primitive continuity of nervous parietes in the brain anterior to (cephalad of) the notochordal axis, together with a more correct evaluation and limitation of the sutura neurochordalis of His. It is felt that there has been given an interpretation also consistent with the actual facts of brain growth and with the pattern of vertebrate ontogeny which was less apparent if the approach was from the neurological side as in the case of the researches of Johnston and Schulte and Tilney. As has been insisted earlier in this paper, the developmental origin of the brain plate and the morphogenesis of the brain are primarily embryological questions inseparable from the pattern of morphogenesis of the head and of the body as a whole.

It is not my intention to consider in any detail the embryologic aspects of the problem in this place; it is, however, advantageous to briefly mention some contributory evidence from the embryological side. Stated succinctly, it is clear that the vertebrate brain arises from the dorsal blastoporic lip which by growth effecting essentially a potential closure of the blastopore, produces an arrangement of material in the neural plate illustrated in figure 2, D, in which the neurochordal suture marks such a line of 'closure,' while the floor plate is a differentiation along this line expressing the primitively bilateral character of the growth of nervous material. Beneath the neural plate the mesoderm and

notochord exhibit a similar arrangement. For a more adequate comparison on this important point figure 6 of my previous paper is here reproduced as figure 5 A while figure 5 B reproduces the plan of arrangement of notochord, preaxial and paraxial

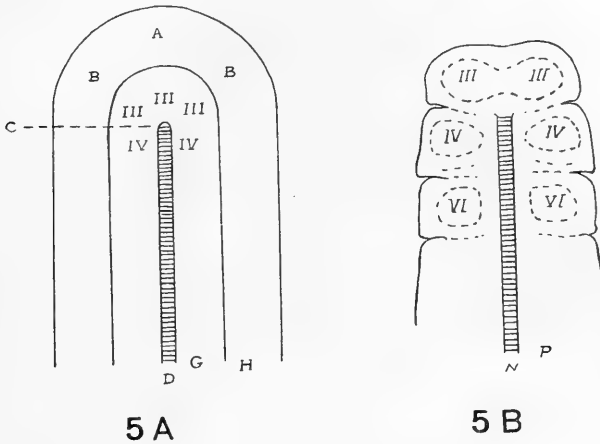


Fig. 5 Schemas comparing the cephalic portion of the neural plate with the underlying notochord-mesoderm relations.

5 A. (Copied from Kingsbury, '20, fig. 6). Diagram to illustrate the interpretation of the cephalic portion of the neural plate. *A*, region of the retinal area(s); *B*, the region of the olfactory lobes (and cerebrum); *C*, cephalic end of the floor plate (and sutura neurochordalis); *D*, the floor plate; *G*, primary motor zone; *H*, primary sensory zone; *III*, nucleus of oculomotor nerve; *IV*, nucleus of trochlearis nerve.

5 B. Portrayal of the relations of notochord and mesoderm, as they exist in a form in which the head cavities are well developed. *III*, premandibular somites and cavities with their prechordal connection and cross canal; *IV*, mandibular somites and cavities; *VI*, Hyoid somites and cavities. The lateral mesoderm and pericardial cavity are of course related, but not indicated.

mesoderm essentially as it would exist in any form (e.g., shark, turtle, certain birds) in which the 'head somites' are well developed. To make the comparison more forceful, the cranial nerve number marks the somite from which its territory of innervation is derived.

The notochord terminates anteriorly in a zone of proliferation where mesoderm and entoderm are for some time confluent. The material in which the anterior end of the notochord terminates, the prechordal plate, is actually or potentially preaxial mesoderm and furnishes the material from which differentiate the muscles innervated by the oculomotor (IIIrd cranial) nerve. The paper of Adelmann, several times referred to, critically examines the growth transformations in this region and furnishes us with an adequate conception of the significance of the prechordal plate. While the prechordal plate is primitively preaxial, its growth is largely bilateral rather than in the median plane, a feature important in understanding the morphology of the head. Thus, if the series of medial planes in *Squalus* (figs. 17 to 26) are compared, it is seen that the preaxial mesoderm which separates off from the entoderm and gains a cavity (connecting the cavities of the premandibular somites), becomes eventually drawn out to the sides and disappears without contributing directly to preaxial median structures, unless indeed the most anterior portion connecting the 'anterior' head cavities may persist (cf. Adelmann, '22).

The bilateral growth appears more precocious in the higher vertebrates, particularly mammals, disguising the significance of primitive relations and rendering an appreciation of the morphogenetic pattern difficult. With the disappearance of medial mesoderm the notochord may through growth attain secondary relations quite different, as fusion with the hypophysis (cf. Rand, '17, et al.). In the chick, likewise, the attenuation of the medial preaxial mesoderm may give rise to faulty interpretations if one were to omit comparison with birds, such as the duck, in which the differentiation is more marked (cf. Adelmann). The slight development of the medial preaxial mesoderm in the chick as compared with the shark may be seen on comparison of the two sets of median planes, plates 2 and 3. It should be appreciated, I think, that we are dealing with a mode of differential growth possessing marked plasticity, hence interpretation in terms of a rigid morphology is not adequate.

The bilateral growth characteristic of the mesoderm is clearly also a marked feature of the growth of the neural plate and is indicated in the comparison of figures 6 to 10 particularly. Also, it is precociously so in chick and mammals, and this is unquestionably a factor in the difficulty with which the primitive relations are recognized.

The interpretation of the neural plate here presented furnishes also an adequate basis for the understanding of the morphology of the head as a whole. Certain features of cranial morphology that may be better comprehended on the basis of the present interpretation were indicated in my earlier paper, and since it is the intention to review the matter in a subsequent publication, it will not be further discussed here. As far as the brain tube is concerned, the outstanding feature, in addition to the marked bilateral growth already referred to and the well-known foldings of the tube as a whole due to growth, is the great expansion of the dorsal portion, effecting a rotation, as it were, roughly around the region of preaxial mesoderm as a center or axis. This brings, thus, dorsal regions ventral, ventral aspects more dorsal, more caudal material cephalad—thus reversing the primitive sequence, with the more rostral expansion primitively dorsal. Certain of these rotations are more extreme in the embryo than in later stages.

Little need be added to what was previously said upon the effect of the interpretation upon the relations of alar plate, basal plate, and sulcus limitans in the prechordal portion of the neural tube. The boundary between them as primary sensory and motor zones must necessarily be here indefinite and perhaps indeterminate. The boundary clearly lies, I think, in the region of the mammillary recess. The question, however, possesses no embryological bearing and I gladly leave it to the consideration of neurologic workers.

Only as a comment at this time, it may be said that the mode of growth of prechordal neural plate and preaxial and paraxial mesoderm commented on above furnishes us with a suggestive basis for understanding the morphogenesis of the hypophysis.

It is not the purpose of this paper to consider the question, in how far this conception of the brain plate may assist in the understanding of the purely neurologic problem of the structural pattern of the brain, but there is much that is suggestive. The floor of the midbrain, the ganglion interpedunculare, the corpus mammillare particularly, invite examination, while many fundamental structural relations of the vertebrate brain might be mentioned whose review from the standpoint here presented would be indicated. Inasmuch as other aspects of the broad problem of developmental pattern claim the writer's attention, he does not plan, for the present at least, to test further the neurological possibilities of the interpretation, even though a strong curiosity in the matter prompts thereto. It is hoped, however, that the concept may afford a sound basis for neurological investigation.

In closing, I wish to acknowledge the kindness of Prof. S. H. Gage who placed at my disposal the chick and shark embryos⁶ of the collection in his charge, and the helpful coöperation of Mr. H. B. Adelmann at points where my line of investigation crossed with his.

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⁶ Many of these series bear evidence of the attention Mrs. S. P. Gage had devoted to the problem of the anterior end of the neural plate. That she had definitely rejected as not satisfactory the His plan of arrangement of the zones is apparent from her paper of 1905. Doubtless had her health been spared she would have arrived at some alternative explanation, such as that here presented.

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PLATE 1

EXPLANATION OF FIGURES

- Photographs, all at a magnification of ten diameters, except figure 16 which is
- × 8. Embryos of *Squalus acanthias* dorsal aspect.
- 6 Series 42, no somites, length 1.5 mm.
- 7 Series 45, 6 to 7 somites, length 2.2 mm.
- 8 Series not sectioned, length 2.4 mm.
- 9 Series 48, ca. 8 somites, length 2.16 mm.
- 10 Series not sectioned, length 2.18 mm.
- 11 Series 52, 11 to 12 somites, length 2.9 mm.
- 12 Series 54, 13 to 14 somites, length 3.5 mm.
- 13 Series 55, 15 somites length, 4.0 mm.
- 14 Series 59, ca. 17 somites, length 4.8 mm.
- 15 Series 69, 29 somites, length 5.0+ mm.
- 16 Series 75, 41 somites, length ca. 7.0 mm.

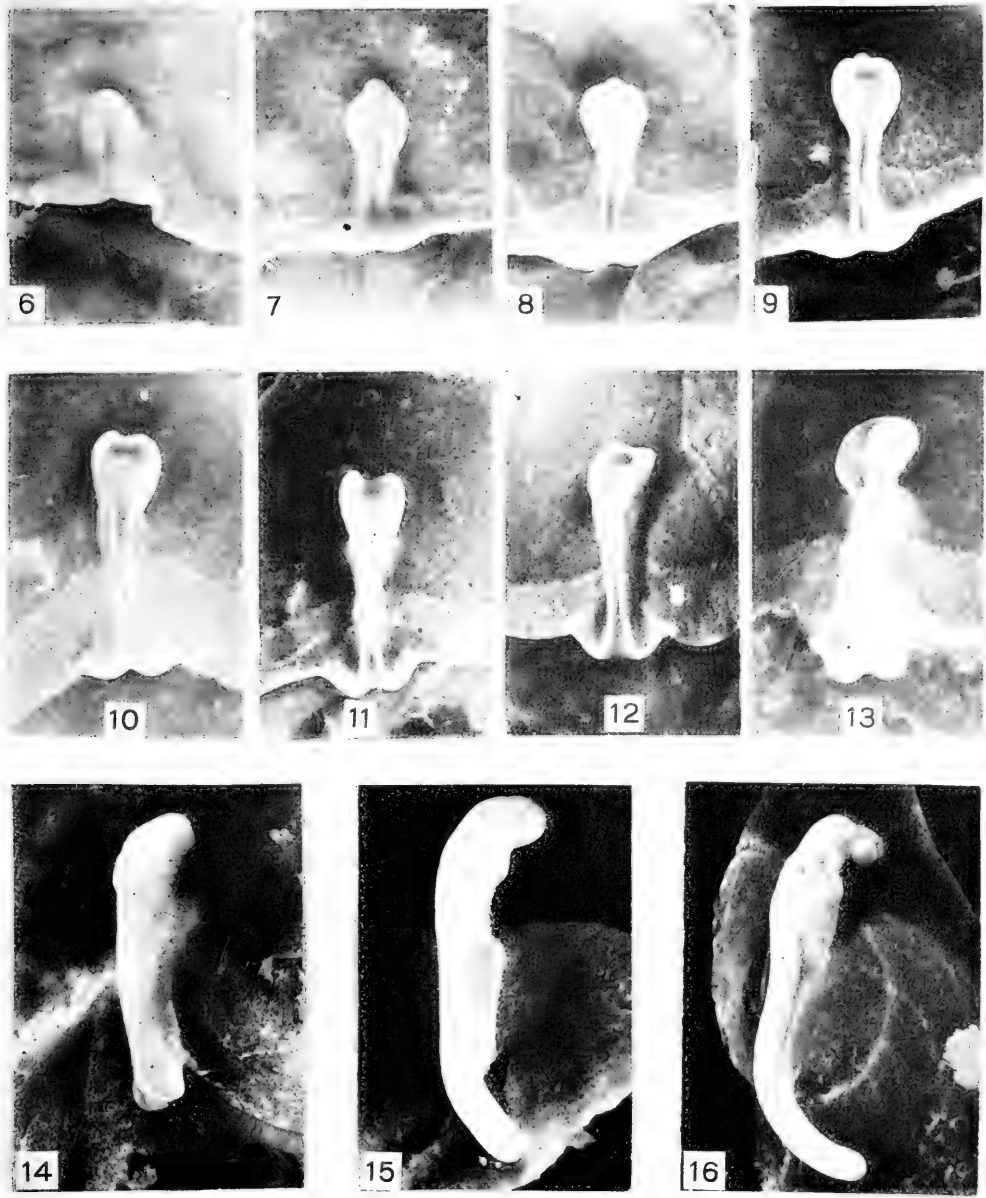


PLATE 2

EXPLANATION OF FIGURES

Median plane reconstructions from sagittal sections of *Squalus acanthias*. The notochord is indicated by cross-barring, the neural plate stippled, entoderm and preaxial mesoderm, stippled or black, ectoderm generally in black. All figures at the same magnification, $\times 25$.

- 17 Series no. 42, no somites, length 1.5 mm.
- 18 Series no. 50, 8 somites, length 2.7 mm.
- 19 Series no. 52, 11-12 somites, length 2.9 mm.
- 20 Series no. 55, 15 somites, length 4.0 mm.
- 21 Series no. 56, 16 somites, length ca. 3.0 mm.
- 22 Series no. 51, length 4.5 mm.
- 23 Series no. 70, 28-30 somites, length ca. 5.0 mm.
- 24 Series Gage 3, length 9-10 mm.
- 25 Series no. 46, length 9-10 mm.
- 26 Series no. 38, length 23 mm.

F, actual or prospective location of the fovea isthmi and the anterior end of the floor plate.

P, Tuberculum posterius (v. Kupffer).

I, 'Tubercle of the floor' (see text).

FUNDAMENTAL PLAN OF VERTEBRATE BRAIN
B. F. KINGSBURY

PLATE 2

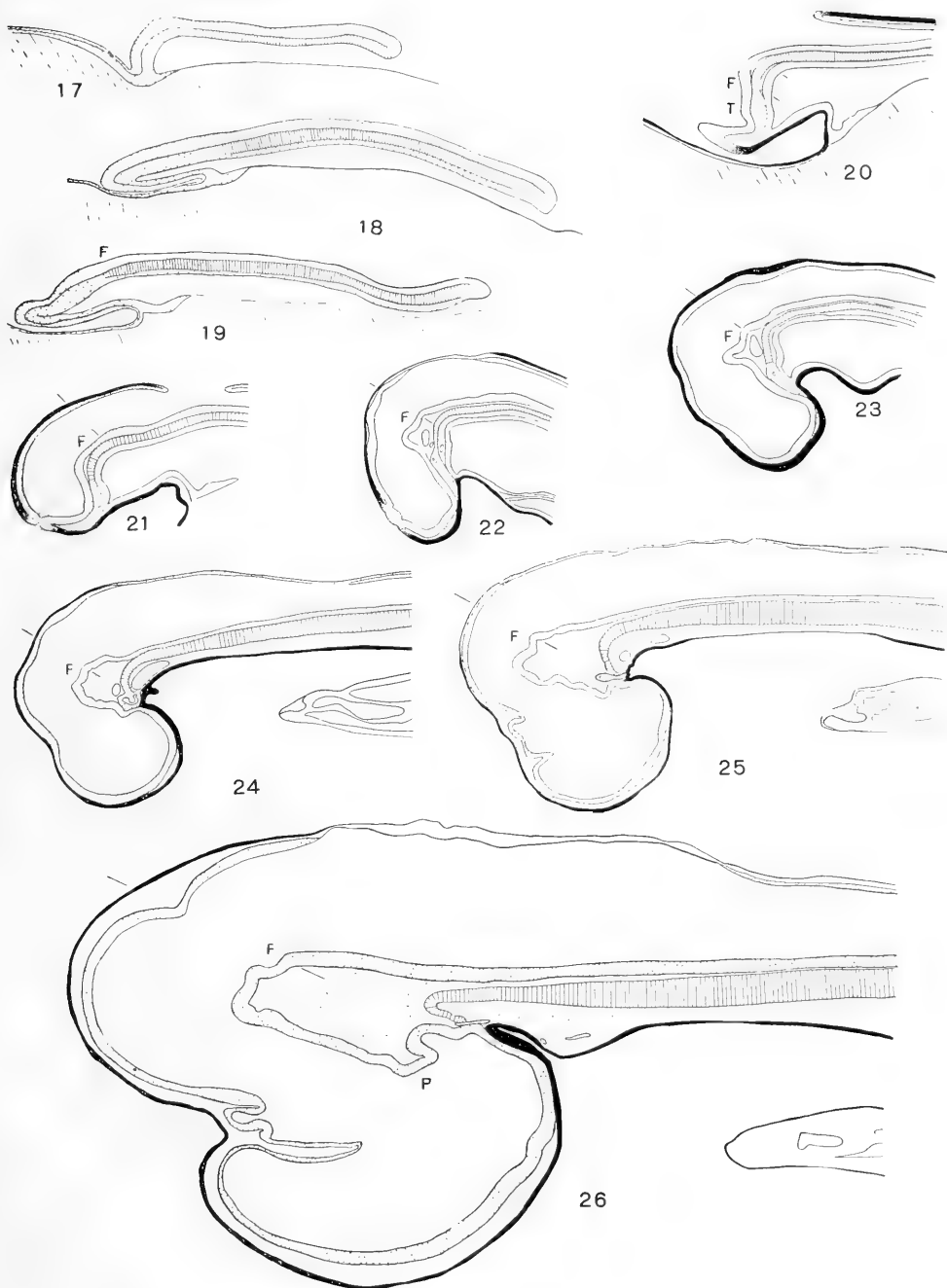


PLATE 3

EXPLANATION OF FIGURES

Median plane reconstructions from sagittal sections of the chick embryo, head region only. The notochord is indicated by cross-barring, the neural plate stippled, entoderm and preaxial mesoderm (prechordal plate) stippled; the ectoderm shown in black. All figures at the same magnification, $\times 50$.

27 Series 139, 6-7 somites.

28 Series 106, 10 somites.

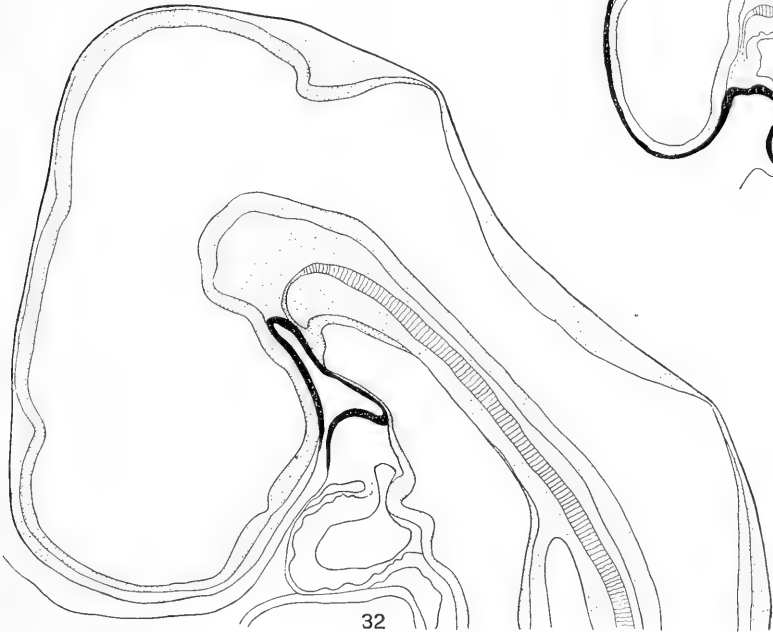
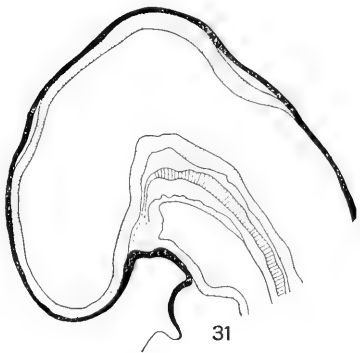
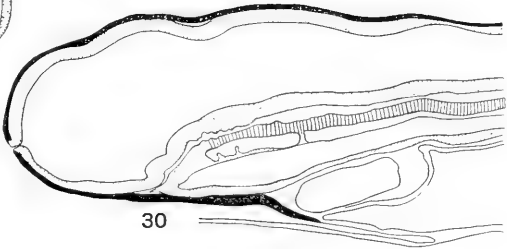
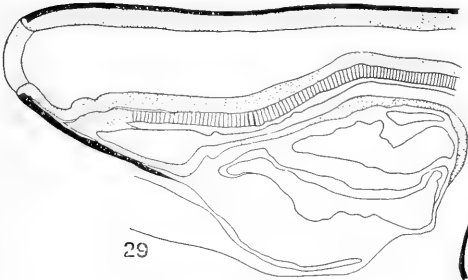
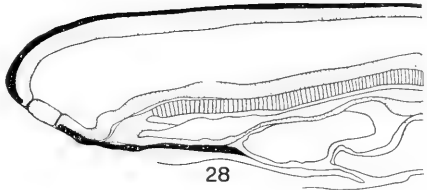
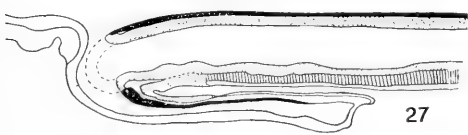
29 Series 119, 14 somites.

30 Series 109, 16 somites.

31 Series 127, 22 somites.

32 Series Gage 54s, 30 + somites.

33 Ventral view of a model of the head of a chick, eight to nine somites, anterior portion, $\times 67$. To illustrate the ventral end of the 'sutura terminalis' marking the anterior end of the brain-plate. Back of it is the 'hypophyseal area' and a shallow Seessel's pocket continuous caudally with a dorsal pharyngeal groove.



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On the afferent paths of the sympathetic nervous system, with special reference to nerve cells of spinal ganglia sending their peripheral processes into the rami communicantes.

In embryos of birds (sparrow) and mammals (pig) there is demonstrated for the first time by direct anatomical observation the occurrence in the spinal ganglia of nerve cells, the peripheral processes of which pass into the rami communicantes. These are the cells of origin of Kölliker's fibers and are regarded as afferent visceral sensory neurons.

ON THE AFFERENT PATHS OF THE SYMPATHETIC NERVOUS SYSTEM, WITH SPECIAL REFERENCE TO NERVE CELLS OF SPINAL GANGLIA SENDING THEIR PERIPHERAL PROCESSES INTO THE RAMI COMMUNICANTES

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SEVEN FIGURES

During the last ten years our interest in studies concerning the sympathetic system has increased progressively, as clinical applications of the knowledge gradually acquired have become manifest. Consequently, there is an increasing number of investigators who carefully attempt to clarify the numerous problems of anatomy, physiology, and physiopathology which, up to the present time, are still under discussion in this intricate field.

Anatomists occupy themselves especially with two subjects of paramount importance. These are: the connections of the sympathetic system and the origin and course of its afferent path. On the other hand, studies of the morphology of the sympathetic ganglion cells have decreased, since the description given by Cajal (1) by the aid of his silver method. Serious contributions to this subject have, however, appeared, of which I may be permitted to recall those of my pupil, Riquier (2), on the morphology of the cells of the ganglion oticum and the junction between spinal and sympathetic ganglia of the turtle.

In order to examine as closely as possible the two problems mentioned, anatomists, who follow to-day a procedure more and more biological, have been less inclined to employ direct observation of pictures which we are able to secure with present methods of staining normal nervous tissues than physiological experimentation and study of the changes produced by injuries to some portion of the sympathetic itself or other nervous structures which are supposed to be in relation with it.

The results which the English and American schools have secured by these methods are so well known that it is quite unnecessary to go into a detailed exposition and to assert that we expect great profit by their further use. Nevertheless, it seems to me that the study of pure anatomical preparations also should be able to give new contributions of value toward the solution of the complicated problems related to the sympathetic system. The researches of Müller (3) and his coworkers who have employed Bielschowsky's method and the Rongalitweiss-staining support my contention, which agrees also with the following findings concerning the question of the sensory sympathetic paths.

Lennander's idea that all visceral pain was mediated through the parietal peritoneum or through the sensory fibers of the visceral blood-vessels has been disproved, we may say, definitely by the majority of physiologists. At the present time two other views are under discussion about the pathways which convey visceral sensibility. The one, to-day widely accepted, assumes that all afferent sensory fibers present in the sympathetic nerves are peripheral processes of neurones the cell bodies of which lie in the spinal ganglia. Such special dorsal root neurones are believed to send their processes, via rami communicantes, into the sympathetic trunks, where they meet with and accompany the efferent postganglionic fibers to their terminations in tissues. According to such a view, visceral sensibility would be conveyed in pathways similar to those of the varieties of common sensibility, at least so far as concerns the afferent impulses of visceral origin which appear in consciousness. That is, sympathetic sensory structures are denied and it is affirmed that all the sympathetic elements are of motor character. The opposing view, supported by Dogiel and nowadays defended by the minority of neurologists, recognizes in the sympathetic mechanism sensory neurones which belong primarily to this system. These neurones would have their trophic centers in the various autonomic ganglia; their peripherally directed processes end in sensory terminal apparatuses of the viscera; their centrally directed processes run through the rami communicantes into the spinal ganglia

where they form terminal arborizations about some spinal ganglion cells, chiefly those of Dogiel's type II. The visceral impulses conveyed by these neurones are collected from spinal ganglion cells, the centripetally directed processes of which transmit them through the spinal cord upwards. Besides this long path, Dogiel assumes a shorter one which conveys those afferent visceral impulses which never rise into consciousness at all, but expend themselves in the production of peripheral reflexes; the centripetal fibers of many sensory autonomic cells are believed to end within sympathetic ganglia in connection with the dendrites of efferent cells. It will be here noticed that Dogiel's view does not necessarily deny the presence of afferent cerebro-spinal fibers in the viscera.

It is not my purpose to cite all the discussions about the two foregoing views, discussions which we meet in every modern book about the sympathetic system, nor to recall the modifications of Dogiel's theory, which deserve more than a passing notice. The aim of my paper is merely to call attention to a definite anatomical finding which, in my opinion, gives a very valuable confirmation of the first view, but could not be used to disprove entirely the second.

The theory which assumes that the nerve cells from which arise the afferent paths of the sympathetic system lie in the spinal ganglia is based on physiological experimentation and on the following anatomical facts.

A. Morphological researches of Kölliker and Ramón y Cajal. These investigators have found some large nerve fibers coming off from the spinal ganglion and entering the ramus communicans. Kölliker assumed that these fibers formed the sensory sympathetic paths. Upon reading all of the literature available to me, I could not find that Kölliker or any other investigator has described the nerve cells from which arise those fibers which are commonly called Kölliker's fibers. In the account which Redlich wrote about Kölliker's lecture (4) we read: "*jene freilich unklaren Empfindungen die wir normal von dem Zustande der vom Sympaticus versorgten Teile haben, leiten von der Funktion einer geringen Zahl dunkelrandiger Nervenfasern ab, die von*

den sensiblen Wurzeln der Rückenmarksfasern durch die Verbindungsäste in den Grenzstrang des Sympaticus übertreten." When we consider these words and the influence that at such a time was exerted by the well-known experiments of C. Bernard and Bidder, we cannot but conclude that Kölliker not only was ignorant of the cells from which his large fibers take their origin, but also that he had not definitely disproved that they proceed from the spinal cord through the dorsal roots and traverse the spinal ganglia, as many workers believed. In his textbook Kölliker (5) assumes the existence of fibers which come off from the spinal ganglia, go through the rami communicantes into the sympathetic ganglia and continue to the periphery, but he does not say anything about the ganglion cells in which these fibers have their origin; only in his schema—reproduced, more or less modified, in all textbooks—does he picture them as arising from spinal ganglion cells of the common type.

Cajal (6) writes: "Certains auteurs, et parmi eux Kölliker, pensent que des fibres sensibles, nées dans les ganglions rachidiens, pénètrent dans le sympathique avec les rameaux communicants blancs et ne font que traverser les ganglions sympathiques pour se terminer aux surfaces des muqueuses. Nous avons vu, nous aussi, dans l'embryon de poulet, des fibres épaisses, nées du ganglion rachidien voisin, entrer dans les ganglions du sympathique; mais le fait s'est présenté si rarement qu'il nous a été impossible d'étudier l'origine réelle et la terminaison de ces fibres."

Here reference might be made to the fact that Lenhossék (7) has shown that some fibers from the sensory roots enter the sympathetic ganglia, also that the sphenopalatine ganglion receives a bundle of nerve fibers from the gasserian ganglion, and further that some of the peripheral fibers of the geniculate ganglion enter the chorda tympani. Huber (8) believes that these sensory fibers do not end in the ganglia, as Lenhossék inclines to assume, but pass through the ganglia and become associated with the efferent sympathetic nerves. Huber met such medullated fibers which are larger than the preganglionic fibers in the frog's

bladder and traced them through two and through three small ganglia of the same bladder.

I did not succeed in finding in the literature anything more definite; therefore I believe that we are authorized to come to the conclusion that till now descriptive anatomy has not specified the origin of the so-called Kölliker's nerve fibers.

B. Experimental researches (Scaffidi (9)) show that injuries to the thoracic sympathetic ganglia led to distinct alterations of the stainable substance in the cells of the corresponding spinal ganglia, especially in some cells lying in the peripheral portion of the same.

C. Experimental researches demonstrate that in the rami communicantes albi are to be found large medullated fibers which fall into secondary degeneration only when cut off from the corresponding spinal ganglion, where consequently we must look for their trophic center.

Roux (10) cut, in the cat, anterior and posterior roots in the proximal tract (between spinal ganglion and spinal cord) and stated that many medullated fibers were to be found undegenerated in the corresponding ramus communicans. He further routed out the spinal ganglion and verified the degeneration of these fibers. From his own researches he came to the conclusion that the nerve cells from which the fibers mentioned arise are located in spinal ganglia.

Scaffidi (9) arrived at similar results and conclusions from analogous experiments, substituting, however, for the extirpation of the spinal ganglia a less injurious, more appropriate, operation. After having confirmed that cutting the proximal tract of the dorsal nerve root and the anterior root never produces degeneration of all of the medullated fibers of the ramus communicans, he cut the dorsal root caudad, viz., peripheral to the corresponding spinal ganglion, and so was able to see the degeneration of all the medullated fibers of the ramus communicans.

Of late Ranson and Billingsley (11), to whom the paper of the Italian investigator seems to have been unknown, performed similar experiments, but studied the preparations also by the

pyridin-silver method of Ranson. So far as their researches concern the question of which we speak, we find the experiments described on pages 450 and 451 of their work highly interesting, because the authors give exhaustive and exact description of our medullated fibers and also establish the fact that we meet also afferent fibers without myelin sheath. On the ground of their experiments, Ranson and Billingsley conclude that the section of thoracic spinal nerve roots proximal to the spinal ganglia results in a degeneration of all of the preganglionic autonomic fibers in the corresponding white rami, but leaves the afferent fibers intact, and that, on the contrary, the latter fibers degenerate after section of the nerve distal to the corresponding spinal ganglion. Only in one case, after section of the tenth thoracic nerve distal to the spinal ganglion, the authors found a half-dozen normal myelinated fibers in the corresponding white ramus, but they suppose that these may belong to a small gray ramus accompanying it.

Certainly, in view of what we know about wallerian degeneration, we should be obliged to conclude from these researches that the Kölliker's fibers take origin from cells of the spinal ganglia: but we would not be able to state whether these cells are of common or of special type. Furthermore, one could advance an hypothesis which might be considered too hazardous and perhaps strange, but which could not be entirely disproved. The studies of Langley and Graupner (12) have taught that we meet nerve fibers belonging certainly to the sympathetic system which are covered by an evident myelin sheath; therefore, one could think that those nerve fibers of the white rami which remain intact after cutting the dorsal root proximal to the ganglion and also the anterior root might be sympathetic postganglionic fibers entering the corresponding spinal ganglion with blood-vessels and coming off through the rami communicantes.

In sum, we express the opinion that Kölliker's view is strongly supported by the researches stated under A and B, but we could not deny that doubts, already expressed by Onuf and Collins (13), are still vexing the minds of investigators. Therefore, we must consider of paramount importance further researches which

would tend to clarify the relations of these fibers to their cells of origin by means of direct anatomical findings. In accordance with these conclusions, I find it interesting to report the following results which, after long work, I was able to reach by Golgi's method in successfully impregnated specimens.

Series I. Findings in bird embryos by the rapid Golgi method

Figure 1 reproduces a part of a cross-section of an embryo of *Passer sardoa* near the time of hatching. We see a bipolar cell of a thoracic spinal ganglion (*SP.G.*) sending its peripheral process into the ramus communicans (*R.c.*), where it can be traced as far as to a formation which by its position, characters, and presence of a multipolar nerve cell we can easily recognize to be a sympathetic ganglion (*Sym.G.*).

In figure 2 is reproduced, from a cross-section of the thoracic part of an embryo of *Passer sardoa*, the anterior lateral region of the spinal cord (*Sp.C.*) and a spinal ganglion (*SP.G.*). In the spinal ganglion three nerve cells (the more darkly pictured) are to be found which send their peripheral processes into the ramus communicans (*R.c.*), while two other cells (drawn more lightly) send their processes towards the peripheral nerve (*Per.N.*).

The same evidence is to be seen in figure 3 and concerns one of the three nerve cells stained by Golgi's method (the one marked with a cross). In this preparation another interesting finding appears: one fiber (*An.R.F.*) of the anterior spinal root divide at the level of the ramus communicans (*R.c.*) and sends one branch into the ramus communicans and the other into the peripheral nerve (*Per.N.*). Another spinal ganglion cell sending its peripheral process into the ramus communicans is reproduced in figure 4.

Series II. Findings in pig embryos by the Golgi method

Figure 5 reproduces part of a cross-section of one embryo. One spinal ganglion cell (*SP.G.*) is clearly seen sending its peripheral process through the ramus communicans (*R.c.*) as far as the sympathetic ganglion (*Sym.G.*). Another nerve cell sends its peripheral process into the peripheral nerve (*Per.N.*), where it meets the fibers of the anterior spinal root. A similar finding is to be found, under greater magnification, in figure 6.

It is not necessary to emphasize the chief result of these researches. Though in the birds sometimes it is not easy to identify the ramus communicans by reason of its peculiar topographic relations, yet this is recognized clearly enough in the preparation from which is reproduced the first picture. Besides, I have met the same evidence in mammalian embryos.

Supported by my findings, of which I have pictured the most evident, the results afforded by my studies may be summarized as follows: *In embryos of birds and mammals there is demonstrated for the first time by direct anatomical observation the occurrence in the spinal ganglia of nerve cells the peripheral processes of which pass into the rami communicantes.*

In my own preparations there is no evidence which could demonstrate that these nerve cells have peculiar character or position. One could perhaps question the constancy of such findings. I do not believe that they are occasional because in thirty well-impregnated bird embryos I met them more or less clearly fifteen times, and in thirteen pig embryos six times.

That previously illustrious investigators, such as Kölliker and Cajal, though successfully impregnating the processes, have not had the good fortune to impregnate their cells of origin, we can easily understand when we recollect all the circumstances which cooperate to modify the results of the wonderful method of Golgi, which is certainly able to give new and interesting findings about the relations of nerve cells when applied carefully and with sufficient persistence.

Fig. 1 Cross-section of embryo of *Passer sardoa*. Golgi's method. $\times 87$. A spinal ganglion cell is seen sending its peripheral process into the ramus communicans. *R.c.*, ramus communicans; *Sp.C.*, spinal cord; *SP.G.*, spinal ganglion; *Sym.G.*, sympathetic ganglion.

Fig. 2 Cross-section of embryo of *Passer sardoa*. Golgi's method. $\times 267$. Three nerve cells of the spinal ganglion send their peripheral processes through the ramus communicans. *Per.N.*, peripheral nerve; *R.c.*, ramus communicans; *Sp.C.*, spinal cord; *SP.G.*, spinal ganglion.

Fig. 3 Cross-section of embryo of *Passer sardoa*. Golgi's method. $\times 267$. The spinal ganglion cell marked with a cross sends its peripheral process into the ramus communicans. One fiber of the anterior root (*An.R.F.*) divides at the level of the ramus communicans; one of the branches runs through the ramus itself. Other indications as in figure 2.

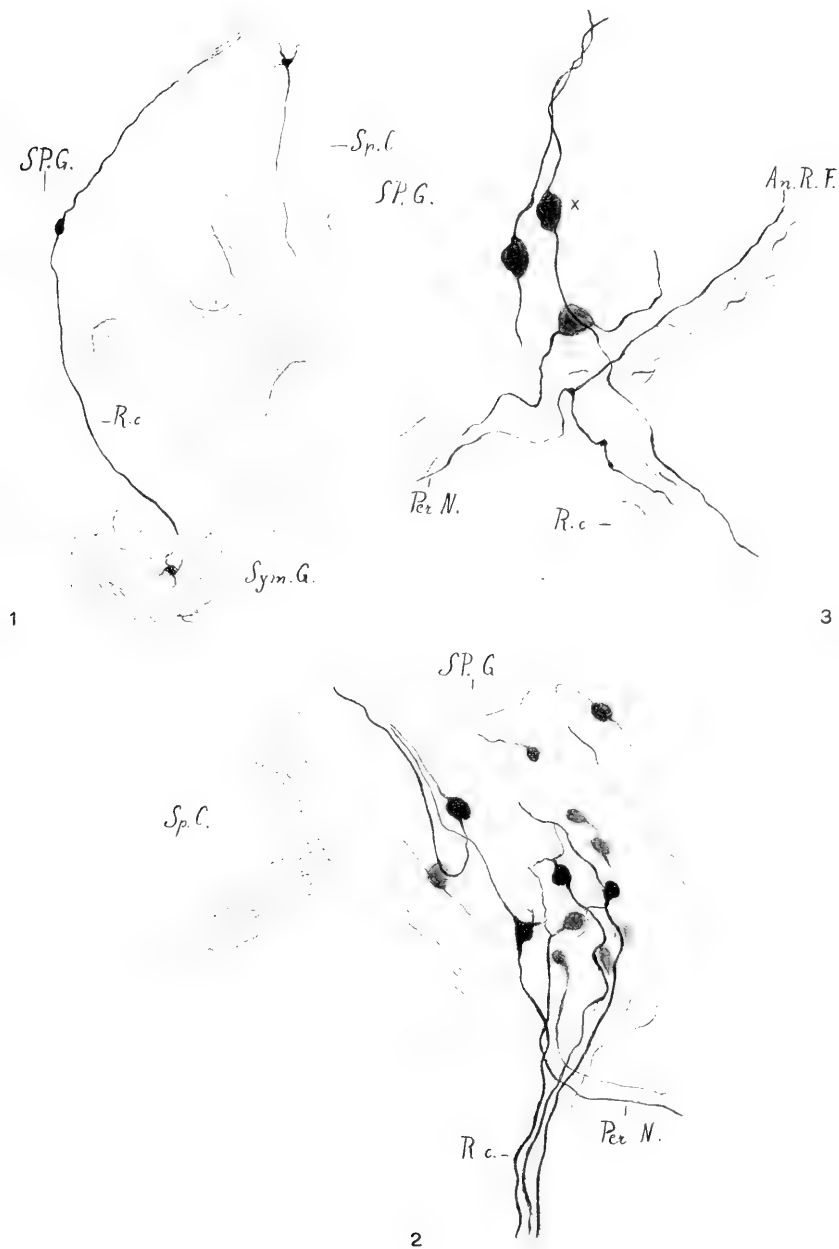




Fig. 4 Cross-section like that shown in figure 3. $\times 410$. Spinal ganglion cell giving off peripheral process to the ramus communicans.

Fig. 7 Cross-section of embryo of *Passer sardoa*. Golgi's method. $\times 120$. *D.B.P.N.*, dorsal branch of the peripheral nerve; *R.c.*, ramus communicans; *Sp.C.*, Spinal cord; *SP.G.*, spinal ganglion; *Sym.N.C.*, sympathetic nerve cell the axon of which, running through the spinal ganglion, reaches the dorsal branch of the peripheral nerve.



Fig. 5. Cross-section of pig embryo. Golgi's method. $\times 267$. The upper spinal ganglion cell shows its peripheral process going through the ramus communicans as far as the sympathetic ganglion. *Per.N.*, peripheral nerve; *R.c.*, ramus communicans; *Sp.C.*, spinal cord; *S.P.G.*, spinal ganglion; *Sym.G.*, sympathetic ganglion.

Fig. 6 Cross-section of pig embryo. Golgi's method. $\times 333$. The ganglion cell pictured on the right side gives off a peripheral process entering the ramus communicans. Indications like those of figure 5.

The support which my findings give to Kölliker's view of the afferent sympathetic paths is clear. But they are not sufficient to disprove the possibility of the existence of other sympathetic sensory paths. First, it is not certified by my observations where the peripheral processes arising from the described spinal ganglion cells end. Second, reference may also be made to some recent investigations performed with the method of secondary degeneration and by direct anatomical observation which seem to demonstrate the occurrence of afferent sympathetic fibers the trophic centers of which lie in the sympathetic ganglia while the processes run through the rami communicantes and, according to many workers, reach the spinal ganglia. Here it will be noticed that Ranson and Billingsley found, in cat XIV, after section of the tenth thoracic nerve distal to the spinal ganglion, a half-dozen normal myelinated fibers in the corresponding white ramus, but they do not think that they are afferent fibers, the cells of origin of which lie in the sympathetic ganglion. On the contrary, they believe that these may belong to a small gray ramus accompanying the white. It must also be mentioned that Müller, who saw some sympathetic fibers entering the spinal ganglion, supposed that they are postganglionic sympathetic fibers running through the ganglion to go off with the dorsal branch of the peripheral nerve. Such a view is supported by my finding reproduced in figure 7 (part of a cross-section of an embryo of *Passer sardoa*), where is shown a sympathetic nerve cell (*Sym.N.C.*) sending its postganglionic process through the corresponding spinal ganglion (*Sp.G.*) into the dorsal branch of the peripheral nerve (*D.B.P.N.*), to which runs also a fiber coming off from the anterior spinal root.

Finally, in the problem of visceral sensibility the anatomists must not forget the recent physiological investigations of Lehmann, who believes that the visceral sensory fibers run above all through the anterior spinal roots.

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